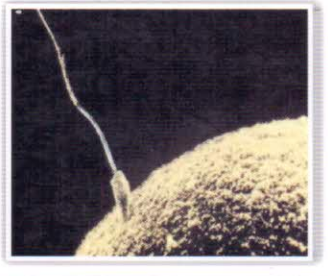
REPRODUCTIVE PHYSIOLOGY OF DOMESTIC ANIMALS

Dr. Muhammad Subhan Qureshi



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REPRODUCTIVE PHYSIOLOGY OF DOMESTIC ANIMALS

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Dedicated to

My mother

Who allowed my involvement in science and

Prof. Dr. M. Amjed

Who supported my scientific Endeavours

PREFACE

This textbook has been written to meet the requirements of the graduates and postgraduate students of the local universities. The available literature on the subject of reproductive physiology in domestic animals has been reviewed. Mental caliber of the local students was observed during the last two years and the materials of this monograph are made understandable for the students. The available literature in animal reproduction has been written by foreign authors, mostly covering the subject very extensively, making it difficult for the beginners. In addition no single available book can cover the whole syllabus, especially of Reproductive Physiology in DVM degree programme. As the graduate students have to cover many subjects during a semester, they can not run after many books to understand a phenomenon. So availability of a comprehensive and current manuscript was considered essential for meeting requirements of the students of animal reproduction.

This manuscript has been prepared by going back to the approved syllabus contents. Systemic coverage of each topic has been made, starting from definitions and basic explanations of the topic. The succeeding paragraphs provide a revision of current state of knowledge on the respective topic, and field application of the knowledge so gained. Explanation of various biological mechanisms and processes has been made in a simple but comprehensive style using the information that are agreed by most authors and which are in use in the scientific community of the region. Graphic illustrations and tabulation are presented for explaining various biological phenomena, laboratory protocols and therapeutic intervention strategies.

Field application of the science has been reported along with all the relevant sections. Cost-effectiveness, compatibility with the international standards and adoption of various reproductive technologies relevant to the subject materials, are explained. Personal experience of the author in semen processing, factors affecting and managing reproduction under field conditions, and hormonal profiles during various reproductive states have been accommodated in the contents of this monograph.

Dr. RD Geisert, Animal Sciences Centre, Columbia, MO, granted permission to use his illustrations in this manuscript which is highly acknowledged. Cooperation of the senior faculty especially of Prof. Dr. F. R. Durrani, Prof. Dr. G. Habib and Prof. Dr. N. Ahmad is acknowledged. Prof. Dr. M. Amjed has actively supported this project and Prof. Dr. S. K. Khalil has always been commending these endures. Dr. Hamayun has reviewed some portions of this manuscript. Sponsorship of the Higher Education Commission in reviewing, financing and publishing this manuscript is highly acknowledged.

I would not have been unable to do this job without the prayers of my mother, continuous untiring moral support of my wife and my children who made me comfortable whenever I got fatigued and exhausted.

Interest of the students of the BSc (Hons) AH and DVM degree at this University and their inconvenience in accessing desirable literature, has persuaded me to take and complete this task. They have been taught contents of this manuscript during the last two years with productive feedback. I wish that this manuscript prove helpful in meeting their academic needs.

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O people! if you are in doubt about the raising (the resurrection or revival of the body of all human beings on the Day of Judgment), then surely We created you (man and other mammals) from dust (Adam was born of mud), then from a small seed (fish like sperm and ovum leading to fertilization), then from a clot (leach like embryo on 15th day and ends on the 23rd or 24th day), then from a lump of flesh (chewed like embryo, having somites or tissues from which organs will develop, on the 24th or 25th day on top of the scapula, sex differentiation taking place), complete in make and incomplete, that We may make clear to you; and We cause what We please to stay in the wombs till an appointed time (implantation and gestation in uterus for a fixed period), then We bring you forth as babies (parturition), then that you may attain your maturity (onset of puberty and sexual behavior); and of you is he who is caused to die, and of you is he who is brought back to the worst part of life (senility and associated changes events like Alzheimer's disease damaging many areas of the brain, showing characteristic patterns of damaged neurons, producing a progressive dementia characterized by failing attention and memory, loss of mathematical ability, irritability, and poor orientation in space and time), so that after having knowledge he does not know anything; and you see the earth sterile land, but when We send down on it the water, it stirs and swells and brings forth of every kind a beautiful herbage (used as human and animals' feed).

(Al Quraan, 22:5)

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CHAPTER-1

ENDOCRINOLOGY

INTRODUCTION

Reproduction is essential for maintenance and increase in the population of all living things including mammalian animals. They must reproduce and give birth to young ones. Reproduction in mammals comprises the production of gametes by the male and female, copulation, fertilization, gestation and parturition. All these processes need certain hormones and their combinations, to complete. Animal reproduction is also called as "Theriogenology".

Endocrinology Endocrinology is the branch of biological science dealing with the study of hormones and their receptors. Hormones are produced by cells of certain organs, enter into the circulatory system and reach the target organ to inhibit or stimulate its function. Various hormones are found in animal's body and the hormones controlling reproductive functions are mainly released by hypothalamus, pituitary, ovaries, testis, uterus and placenta.

Hormonal Assays Hormonal assays are conducted for the study of reproductive endocrinology and fertility of mammals. These assays are used to evaluate clinical status of the animals and investigate the causes of low fertility. Radioimmunoassay (RIA) techniques help in determining the concentration of hormones in the body fluids and tissues which reflect the function of endocrine glands and the target organs. Enzyme immunoassay (EIA) has been evolved as a cheaper and environment-friendly technology replacing RIA which needs more investment and safety protocols.

Clinical Applications Clinical applications of hormonal assays are practiced to diagnose pregnancy, detect or predict ovulation, occurrence of tumors of reproductive organs and integrated research programs in clinical reproductive techniques.

PRIMARY HORMONES OF REPRODUCTION

Primary hormones of reproduction are those affecting reproductive processes directly. They include hormones from various endocrine glands including the hypothalamus, pituitary, gonads, uterus and placenta. These hormones stimulate or inhibit the activities of the target organs and the target organs impose a positive or negative feedback on the respective endocrine gland. Primary hormones produced by various organs include:

1. Hypothalamus
    a. Gonadotrophin releasing hormone (GnRH)
    b. Oxytocin

c. Prolactin inhibiting factor (PIF)

2. Anterior Pituitary
    a. Interstitial cells stimulating hormone or luteinizing hormone
    b. Follicle stimulating hormone
    c. Prolactin
3. Placenta
    a. Placenta in primates produces human chorionic gonadotrophin
    b. Placenta in equines produces pregnant mare serum gonadotrophin
    c. Placental lactogen
    d. Protein B
4. Ovary
    a. Estrogen
    b. Progesterone
5. Testis
    a. Testosterone
6. Uterus
    a. Inhibin
    b. Relaxin
    c. Prostaglandin F2α

HYPOTHALAMUS AND PITUITARY GLAND

Hypothalamus or "brain of the brain" is situated located beneath the brain on the anterior side. It comprises bilaterally paired nuclei separated by the medial portion of the hypothalamus called as third ventricle. Hypothalamic hormones and their action on pituitary glands have been enlisted in table 1.1. Homeostasis (stable state of equilibrium of the body) is maintained by the hypothalamus. The behavioral changes related to drinking, food intake, general arousal, anger, violence discomfiture escape from danger, enjoyment and copulation.

The hypothalamus regulates numerous functions of the body, while its size comprises such a small amount of tissues. It seem to stimulate the parasympathetic nervous system, decreases the blood pressure and pulse rate, control of digestion and absorption of nutrients from the gastro-intestinal tract. The functions are executed by the lateral and anterior parts of hypothalamus. Stress symptoms like increased rates of pulse and breathing, increased blood pressure, provocation, horror and annoyance are controlled by the medial and posterior components of hypothalamus. Pure behaviors are generated by the action of certain groups of cells in these areas.

Reproductive Functions Hypothalamus produces gonadotrophin releasing hormone (GnRH), oxytocin and prolactin inhibiting factor (PIF). Reproductive function of hypothalamus is divided into two control centers: i) the pre-ovulatory center causes

ovulation through secretion of higher levels of gonadotrophins and rupture of the mature Graafian follicle and; ii) the tonic center maintains lower levels of gonadotrophins in blood circulation, supporting folliculogenesis and progesterone levels.

Oxytocin Originates in the hypothalamus and is transported to the posterior pituitary for storage. Vasopressin is similar to oxytocin. These peptide hormones were identified and synthesized by du Vigneaud who received a Noble prize in 1955. Oxytocin is found in all mammals. Vasoticin is the hypothalamic hormone of birds and has both oxytocin and vasopressin like functions.

The corpus luteum of ewe, cow and human also produce oxytocin. Oxytocin causes contraction of uterine muscles, thus accelerating the parturition process or expulsion of placenta. It also helps in gametes transportation in the oviduct through increased contraction frequency. Estrogen increases the responsiveness of smooth muscles to oxytocin. Oxytocin acts on myoepithelial cells surrounding alveoli in mammary glands of cow leading to milk let down and ejection.

Luteinizing Hormones Releasing Hormones (LH-RH or GnRH) is a decapeptide with 10 amino acids as discovered by Schally and Guillmin who got Noble Prize for describing its chemical structure and the mechanism of its action on pituitary gland. LH-RH controls the secretion of lutinizing and follicle stimulating hormones from the pituitary gland. Synthetic LH-RH and anti-LH-RH have been prepared for field application. The synthetic LH-RH is more efficient than the natural one due to its resistance to enzyme degradation and prolonged attachment with the receptors. Another common term used for the hormone is GnRH.

Other hypothalamic hormones are thyrotrophin-RH, somatostatin or GH inhibition hormone, growth hormone-RH and corticotrophin releasing hormone.

GnRH-I As well as its isoforms exert various types of actions on the target organs. These actions are neuroendocrine, neuromodulatory and neurotransmitter in nature. The reproductive system is controlled by the neuroendocrine axis, while the GnRH-I coordinates the internal and external signals of the nervous system and regulate the reproductive functions of the body.

The invertebrates are exposed to a variety of environments. The GnRH may have got a role helping in survival of these species under unfavorable environment. The GnRH-I down-regulate the nearby genes for various protein-tyrosin phosphatases reflecting the neuro-modulatory role of the GnRH-I. This hormone provides functional linkage among various molecular mechanisms which provides a diversified function to it.

Neurons in the hypothalamus secrete a substance called Cocaine- and amphetamine-regulated transcript (CART) which has been found to be neuropeptides. The mRNA for CART was isolated from striatal extracts and its secretion was enhanced by cocaine and amphetamine. CART has been reported to be expressed in higher concentrations in hypothalamus. The abundance of CART expression in the hypothalamus has led to the conclusion that CART is an mRNA the expression of which ranks third at the level of the hypothalamus. The concentration of mRNA in the hypothalamus correlates with the concentration of CART protein.

PITUITARY GLAND

Pituitary gland is situated at the base of hypothalamus in a bony depression of the sphenoid bone called sella tercica. Pituitary gland is composed of two components. The anterior pituitary is called as adenohypophysis while the posterior pituitary is called as neurohypophysis.

The pituitary gland is a stalk of tissues like a golf club, working under the influence of the hypothalamus. The pituitary regulate the function of thyroid gland. The thyroid gland maintain the body functions in idle state by secreting the thyroid hormone. In addition; the pituitary gland controls secretion of adrenaline from the adrenal gland exhibiting stress symptoms and reproductive functions are maintained through the testes and ovaries.

Anterior pituitary produces growth hormone (STH, somatotrophin or somatotrophic hormone), affecting protein metabolism, controlling body growth and fetal growth. Adrenocorticotrophic hormone (ACTH) controls the functions of the adrenal cortex and thyroid stimulating hormone controls the functions of the thyroid gland.

Pituitary Gonadotrophic Hormones are follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin. FSH and LH are glycoprotein and prolactin does not contain carbohydrates.

FSH secreted by the anterior pituitary gland, has also been termed as follicotrophin. Half life of FSH is 2.5 hours and its molecular weight is 32,000 daltons. In the female, FSH stimulates the growth and maturation of follicle at the ovary leading to production of estrogens and estrous symptoms. Estrogen production by FSH needs the presence of LH. FSH is used in embryo transfer programs for superovulation and is commercially available. In males FSH is responsible for spermatogenesis up to the stage of secondary spermatocytes formation.

LH luteotrophin or interstitial cells stimulating hormone has a hald life of 30 minutes with a molecular weight of 30,000. It is responsible for rupture of Graafian follicle on the ovary, caused by the preovulatory LH surge, leading to ovulation. LH is the main hormone responsible for formation of corpus luteum at the site of ovulation on the ovary. In the male the LH stimulates production of androgens by the interstitial cells in the testis.

Table 1.1 Hypothalamic hormones and their action on pituitary gland

Hormone	Abbreviation	Target Organ	Effect on Target Organ
Leutilizing hormone releasing hormone (gonadotrophin releasing hormone)	LH-RH (GnRH)	Pituitary gland (PG)/ ovary	Tonic or pre-ovulatory release of FSH and LH
Growth hormone releasing hormone	GH-RH	PG/liver	Releases GH, stimulating growth
Growth hormone inhibiting hormone (somatostatin)	GH-IH	PG/liver	Inhibits GH and growth
Thyrotrophin releasing hormone	TRH	PG/thyroid	Releases TSH and prolactin
Corticotrophin releasing hormone	CRH	PG/adrenal cortex	Releases ACTH
Prolactin inhibiting hormone	PIH	PG/mam-mary gland	Inhibits prolactin secretion and lactogenesis
Oxytocin	Oxytocin	Uterus/ mammary gland	Contraction

Tonic release Tonic release of LH and FSH maintains their levels at a basal fashion, controlled by negative feed back from the gonads. Tonic levels are needed for formation of the follicle at ovary and spermatogenesis in the testis. ***Preovulatory surge*** of LH and FSH are observed in females prior to ovulation. This surge is stimulated by higher levels of estrogens in the blood circulation, through positive feed back.

ENDOCRINE FUNCTIONS OF OVARIES UTERUS AND PLACENTA

OVARIES

The ovary performs endocrine (steroidogenesis) and exocrine (ovulation) function. Ovary contains a number of follicles and corpus luteum which form after the rupture of follicles.

Corpus Luteum develops at the site of ovulation after collapse of follicle. The inner walls of the follicle develop into macro and microscopic folds that penetrates the central cavity. The cells develop a few days before ovulation. Hypertrophy and luteinization of granulosa cells commence after ovulation. The luteal tissues develop through hypertrophy of lutein cells.

Corpus luteum secretes progesterone through its lutein cells. Increase in the size of corpus luteum is rapid, initially. In cow the increase in size is rapid from day 3 of estrous cycle to day 12, remains constant up to day 16. Than the corpus luteum regress in case of no conception or remains intact until parturition. Color of the corpus luteum is red during developing or functioning phases and yellow or white after regression. A regressed corpus luteum is called as corpus albican and it is like a scar. In buffaloes a mild increase is noted in milk progesterone during the first postpartum month accelerating during the second month, showing a plateau until fourth month following by a rapid increase later on. Corpus luteum is needed for pregnancy through production of progesterone. They alter the ionic permeability of the myometrial cells membranes lowering the excitability. Progesterone acts as a precursor for production of other steroids necessary for pregnancy and regulates milk production.

Luteolysis is the regression of corpus luteum. It is under the influence of prostaglandin ($PGF_{2\alpha}$) secreted from uterus. Prostaglandin is also available in synthetic form as cloprostenol (Estrumate, ICI).

Ovarian Follicles Ovarian follicles are found on the surface of ovary in different stages of development. They contain the oocytes in alive and viable state. Granulosa cells of the follicles assist in maturation of the oocytes. Follicles develop into Graafian follicles under the influence of FSH and ovulate under the influence of LH hormones.

UTERUS

Uterus is an endocrine gland secreting reproductive hormones. Endometrial glands are branched in nature and the lumen is supported with columnar epithelium. They open into the endometrial surface. With the rising levels of progesterone they grow and with the declining levels they regress. During various phases of estrous cycle the endometrial fluid varies in composition and volume. Uterine fluid contains proteins that are necessary for implantation of embryo in the uterine wall and its development into blastocyt.

Volume of uterine fluid and protein synthesis increases at the time of estrus under the influence of estrogens. Bicarbonate ions, proteins and carbohydrates in the uterine fluid stimulate sperm metabolism and supply energy. Uterine fluid also helps in capacitation of spermatozoa. Endometrial protein leads to synthesis of steroid hormone receptors. Uterine milk provides nourishment to the growing fetus after implantation. Uterine proteins have an immunosuppressive effect preventing rejection of the conceptus.

Luteolytic Function of the uterus comprises production of a luteolytic substance which is transferred from utero-ovarian vein to the ovarian artery which reaches the ovary in greater concentrations and causes luteolysis. Prostaglandin ($PGF_{2\alpha}$) is the luteolytic

substance in ewes and cows produced at uterus and transferred to ovaries through veno-arterial pathways where it causes regression of the corpus luteum.

Intramuscular or intrauterine administration of prosta-glandins also causes regression of corpus luteum. Intrauterine doses of 25 to 30 mg of prostaglandin cause decline of progesterone levels and induction of estrus within three days in most treated cows.

PLACENTA

Placenta produces various hormones found in the mammalian reproductive system. These are Pregnant Mare Serum Gonadotrophin (PMSG), Human Chorionic Gonadotrophins (hCG), Placenta Lactogen (PL) and Protein B.

PMSG is a glycoprotein with α and β subunits, similar to LH and FSH but with a higher content of carbohydrates (sialic acid), which prolongs the half life of PMSG up to several days. Molecular weight is about 60,000 daltons. PMSG injection has a biological effect on the target organ up to a week. PMSG is secreted by equine endometrial cups.

In mares endometrial cups are formed at 40^{th} day of pregnancy and remain up to 85^{th} day. Their FSH like function stimulate development of follicles on ovaries. Some of these follicles ovulate due to LH like function of the PMSG but most of them luteinize and secrete progesterone which is needed for maintenance of pregnancy in mare.

PMSG has been used for inducing superovulation due to its FSH like activity in domestic animals. However, due to its prolonged half life the development of follicle and ovulation prevalent over an extended period, makes it non-feasible as a superovulation hormone.

hCG is a glycoprotein consisting of α and β subunits with a molecular weight of 40,000 daltons. The hormone is secreted by syncytiotrophoblastic cells of the placenta of pregnant primate and is found in both blood and urine. It is detectable on 8^{th} day of conception or second day of implantation through sensitive radioimmunoassay.

hCG has both FSH and LH like activity but the LH like activity is predominant. It converts the corpus luteum of menstrual cycle to corpus luteum of pregnancy in human beings. hCG is detected through a routine immunological assay in pregnancy diagnosis in woman. Due to its LH like activity, hCG is used for treatment of cystic ovaries with a dose rate of 5,000 to 10,000 I.U. of hCG. The follicle either ovulates to form a follicle or luteinize and secrete progesterone for 20 days. After that the corpus luteum regresses and another estrous cycle is initiated.

PL is a protein with chemical properties like prolactin and growth hormone. Its molecular weight is 22,000 to 23,000 and is available in blood circulation at the last trimester of pregnancy. It is responsible for conveying nutrients to the fetus and the fetal growth. PL also promotes milk production in dairy cows.

Protein-B is secreted by placenta with a half life of 7 days and has been detected in cow's placental tissues. It is found in blood but not in milk or urine. It perhaps helps in maternal recognition of pregnancy. It is a reliable test for pregnancy in farm animals.

ENDOCRINE FUNCTIONS OF TESTIS

Testis is the essential male generative organ or gonad of domestic animals, which lies in the scrotum along with the epididymis and other structures. Testicular size varies among species, breeds and seasons. Removal of one testis leads to enlargement of the remaining by 98%. Temperature of testis is maintained below body temperature through thermoregulation. During the cold weather the smooth muscles or dartos tunic, cause retraction of the testis towards the body while during the hot weather the testicles are kept away from body.

Development of Testis occurs in the abdomen, medial to the embryonic kidney (mesonephros). In bull, boar, ram and stallion the rete comes to lie centrally in the testis and in man peripherally. Primordial germ cells migrate to gonadal ridge and convert to germinal epithelium of the seminiferous tubule. In the head of the epididymis the mesonephric tubules continues as the efferent ductules. The rest of the epididymis is constituted by the mesonephric duct. In male mammals the paramesonephric duct degenerates.

The surface of the testis is covered by peritoneum of the vaginal process. Beneath it lays the dense fibrous coat called the tunica albuginea. Fibrous septa divide the parenchyma into lobules of coiled seminiferous tubules, which lead into the labyrinthine mediastinal part of the rete testis.

The spermatic cord suspends the testis and it contains comprise blood vessels supplying and draining blood to the testis, in addition to vas deferens collecting semen from the cauda epididymis. The arteries are surrounded by the venous plexus which decrease the temperature of the arterial blood supply to the testis. The inguinal canal provides passage for entrance of the spermatic cord into the abdominal cavity.

Endocrinological Function of testis is controlled by the anterior pituitary through production of LH, also called as interstitial cells stimulating hormones (ICSH). Production of testosterone in larger quantities exerts a negative feedback on LH production. The testosterone or male sex hormone is secreted by the interstitial cells or Leydig cells, which lie between the seminiferous tubules. The hormone is released into the testicular veins and lymphatic vessels. The spermatogenic cells of the tubules divide and differentiate to form spermatozoa. The sustentacular or Sertoli cells of the tubules form a barrier just before puberty that isolates the differentiating germ cells from the general circulation. Castration of the prepubertal male suppresses sexual development. Regressive changes occur in behavior of adult males after castration. In animal production castration is a standard procedure to modify aggressive male behavior and eliminate undesirable carcass quality.

The testis starts their function during the fetal life and control development of male reproductive tract suppressing the female reproductive tract. This occurs at day 42 of gestation through production of testosterone by the Leydig cells.

THYROID AND ADRENAL GLANDS

Thyroid Gland is a highly vascular endocrine gland situated near the thyroid cartilage of the larynx. It secretes thyroxin which increases the rate of metabolism and the release is increased by cold weather and decreased by hot one.

Function of thyroid is controlled by thryrotrophin releasing hormone (TRH) from the hypothalamus which is a tripeptide. TRH controls the function of pituitary gland for secretion of thyroid stimulating hormone (TSH) and prolactin inhibiting factor (PIF). Thyroxin secretion is modulated by photoperiod.

Hypothyroidism has been found as a causative agent of acycliciy in bitch, however, the mechanism has not been understood. It has been reported that TRH administration causes release of prolactin.

Adrenal or Suprarenal Glands are two small endocrine glands situated at the anterior extremities of the kidneys. The cortex secretes androgens (aldosterone) a reproductive hormone; as well as corticosteroids which regulate carbohydrates, sodium and potassium metabolism. Corticosteroids are also associated with growth of mammary glands and lactogenesis.

The adrenal cortex plays an important role in influencing return to estrus postpartum. Adrenocorticotrophic hormone (ACTH) and corticosteroid injections have inhibited ovulation due to suppression of LH. Corticosteroids are also associated with growth of mammary glands and lactogenesis. ACTH administration reduces LH response to GnRH and a delayed LH response to estradiol. These events resembling stress, lead to development of cystic ovaries.

Corticosteroids from fetal adrenal glands cause parturition as a natural process. It leads to conversion of placental progesterone into estrogens, which in turn causes relaxation of cervix and contraction of reproductive tract. ACTH has been used to induce parturition in cows as it cause production of endogenous corticosteroids. Direct administration of corticosteroids can also cause parturition.

MECHANISM OF ACTIONS OF HORMONES

Reproductive hormones are either protein hormones like FSH, LH and oxytocin or steroid hormones like progesterone, estrogen and testosterone. The target organ exhibits certain metabolic pathways which are not found on other organs. The two types of hormones act differently on the target organs.

Protein and Polypeptide Hormones are produced by the endocrine glands and released into blood circulation (Fig 1.1). The hormones attach with the receptors on the surface of the cell. It results in activation of adenylate cyclase which is causes convertion of adenosine triphospate to cyclic adenosine monophosphate and pyrophospate. The cAMP acts on protein kinase and ATP is produced. Protein kinase acts on DNA in the nucleus and release mRNA that releases into the cytoplasm. It leads to release of a protein which regulates release of steroid hormones, releasing into the blood circulation.

Steroid Hormones Steroid hormones are released by ovaries, testis and adrenal glands. Steroids pass through the cell membrane and the cytoplasm and attaches with

the receptor present on the DNA inside the nucleus (Fig. 1.2). The nucleus release mRNA which comes out of the nucleus and cause protein synthesis in the cytoplasm of the cell.

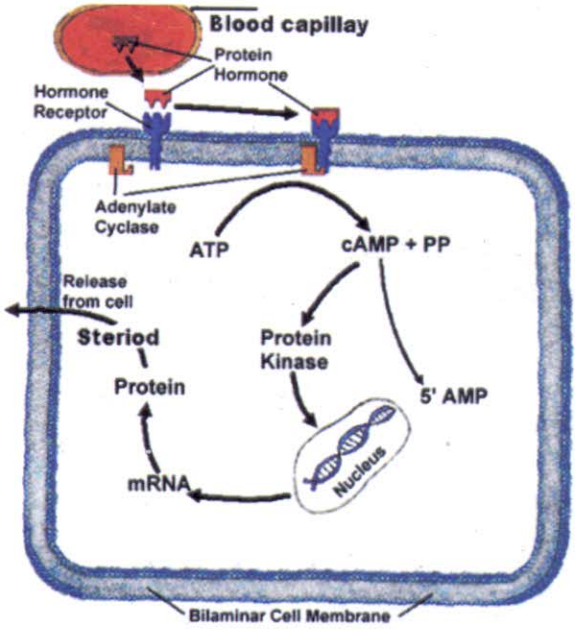

Figure 1.1 Mechanism of action of protein hormones (Hafez, 2000)

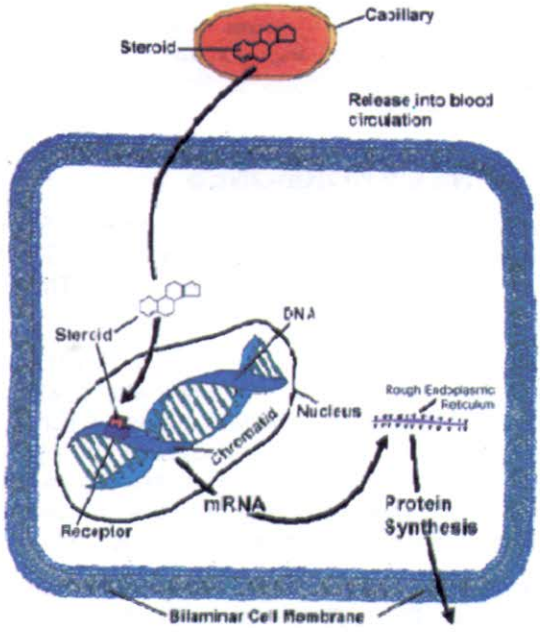

Figure 1.2 Mechanism of action of steroid hormones (Hafez, 2000)

ENDOCRINE CONTROL OF HORMONES RECEPTORS

The receptors of various hormones change quantitatively with the reproductive stage. The number of FSH and LH receptors is probably directly related to the stage of development of follicles and is not under the control of cyclic hormones fluctuation. During the follicular phase in the ewe, when plasma LH levels increase, LH receptors in granulosa and theca cells increase in pre-ovulatory follicles but not in non-ovulatory follicles. In domestic animals and rats, the induction of new binding sites is essentially controlled by FSH. In vitro, FSH induces LH receptors in porcine granulosa cells by unmasking of cryptic FSH binding sites.

THE FEED BACK MECHANISM OF HORMONES

Feedback is the influence of higher levels of some hormones on the endocrine glands controlling their secretion. The feedback may be positive if the action is stimulating and negative if the action is depressing (Fig. 1.3, 1.4).

In Male Mammals the higher levels of testosterone from the Leydig Cells in the testis cause a negative feed back on the action of hypothalamus, thus causing a reduction in the secretion of GnRH from the hypothalamus and FSH and LH from the pituitary glands. In addition the higher testosterone levels also depress function of the pituitary gland, decreasing FSH and LH. Sartoli cells of the testis secrete inhibin which causes a negative feedback on the pituitary gland, depressing its function.

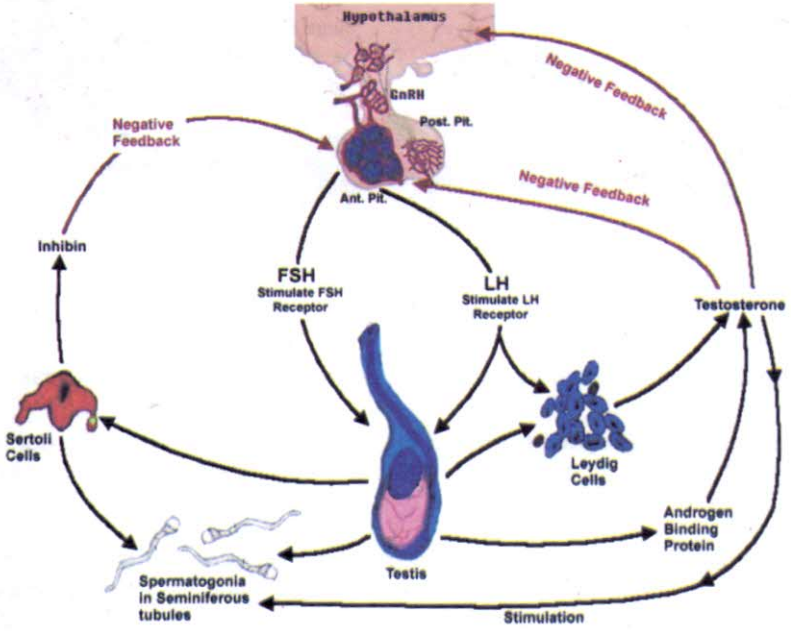

Figure 1.3 Feedback mechanisms of reproductive hormones in males (Geisert, 1999)

In Female Mammals positive as well as negative feedback mechanisms are found. Higher levels of progesterone have a negative feed back effect on the function of

hypothalamus as well as pituitary. During luteal phase the progesterone levels are higher which prevent higher levels of FSH and LH thus suppressing development of dominant follicle and ovulation. The dominant follicle on the ovary during protestrus releases inhibin which has a negative feedback on the pituitary preventing higher levels of FSH and development of more dominant follicles.

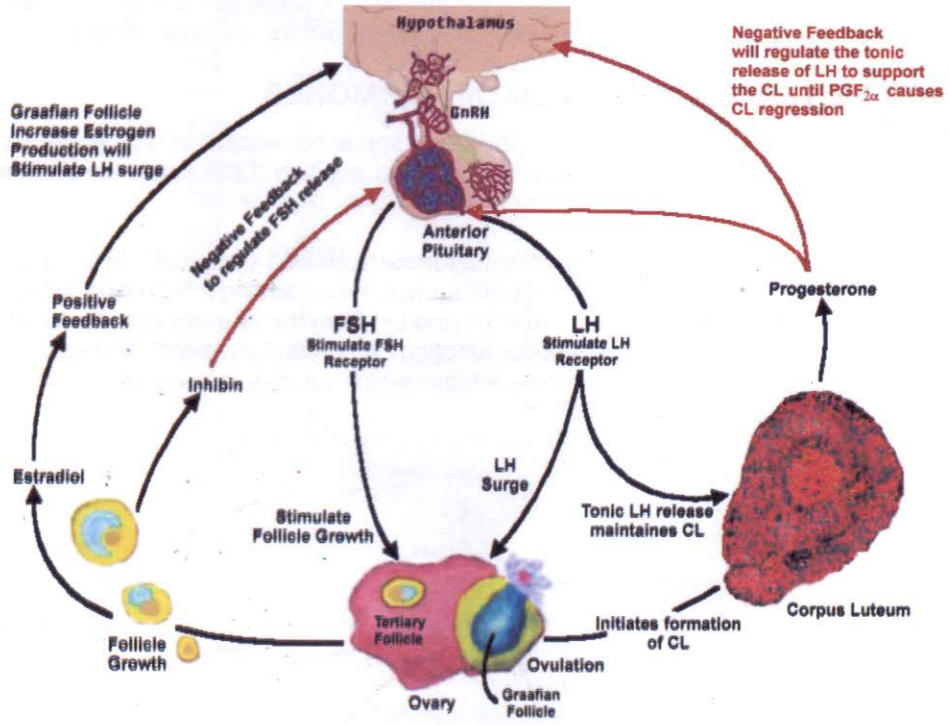

Figure 1.4 Feedback mechanism of reproductive hormones in females (Geisert, 1999)

Higher levels of estradiol from the dominant follicle on the ovary during oestrus, causes a positive feedback on the pituitary leading to enhanced production of LH (called LH surge) responsible for ovulation.

The secretion of GnRH is interfered by changing levels of Estradiol and progesterone. This is accomplished by affecting the secretion of GnRH or influencing the function of cells in the anterior pituitary gland. It has been reported that inhibition of the GnRH pulse amplitudes is caused by the negative feedback actions of E2. P4 exerts its negative feedback action through its effect on the frequency of GnRH pulse frequency.

In heifers the negative feedback of P4 decreased the secretion of GnRH release and also reduced the expression of GnRH mRNA during the mid-luteal phase of estrous cycle. The negative feedback of E2 on episodic release of GnRH was reported as

significantly effective during anoestrus in sheep. The effects of gonadal steroids on the synthesis of GnRH-I is not clearly explainable as compared to the effect on secretion. On one hand the direct effects are demonstrated while on the other, the indirect effects are exerted through steroid-responsive neuronal systems found in different compartments of the brain. The direct effects may be overridden under undesirable conditions like stress and under-nutrition.

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CHAPTER- 2

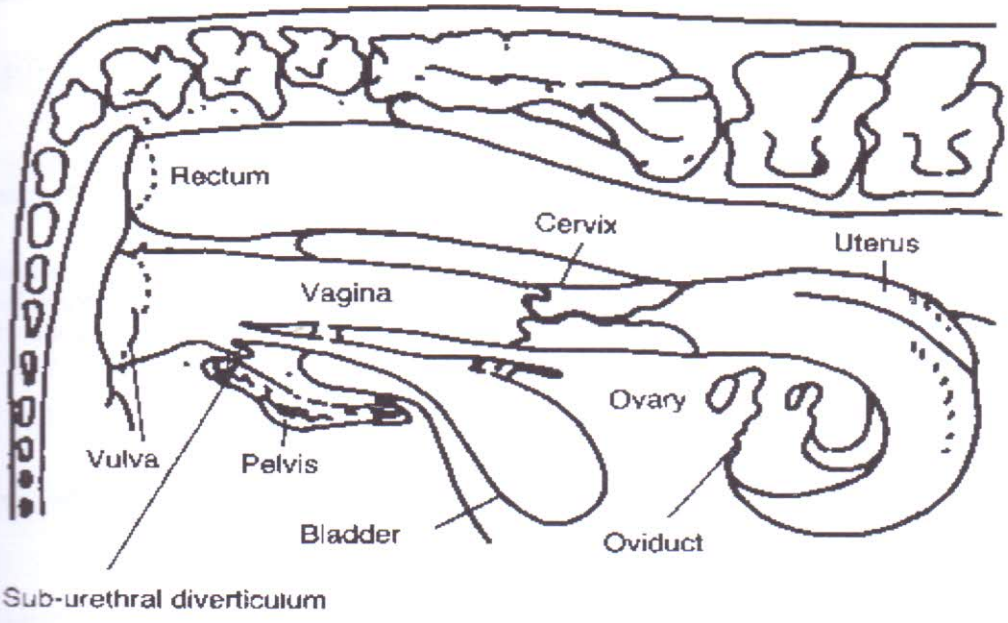
FEMALE REPRODUCTIVE PHYSIOLOGY

STRUCTURE AND FUNCTION OF FEMALE REPRODUCTIVE ORGANS

Female Reproductive Organs are divided into three components: i) ovaries; ii) fallopian tubes; iii) uterus and iv) external genitalia. The reproductive organs are contained in the pelvic cavity at the posterior end of the abdominal cavity. The lateral view of reproductive tract of the cow (lateral view) inside the pelvic and abdominal cavities is shown in Figure 2.1

Obstetrics is the branch of veterinary science which deals with the required assistance during the act of parturition in animals. The cases of animal birth are managed and surgical operations are conducted for management of pregnancy and labor. Rectal palpation is made for monitoring of reproductive status of animals and pregnancy diagnosis, the space being provided by the pelvic cavity for all these procedures.

Fig. 2.1. The reproductive system of cow



Source: Ball and Peters, 2004.

THE PELVIC CAVITY

The pelvis or the pelvic cavity is the smallest and most posterior of the three great cavities of the trunk. The pelvic cavity is important for an obstetrician because it provides the birth passage for delivery of the fetus. It has a rigid wall, and provides a resistance to the passage of the fetus as a result of any variation from the normal presentation. The pelvic cavity is composed of bony and legamentous structures.

The Bony Pelvis is the bony portion of the pelvic cavity and consists of the Os coxae at the sides and below. The upper portion of the bony pelvis is formed by the sacrum and first three coccygeal vertebrae. Each Os coxae is composed of three components:

i) the ilium which is the largest portion of the os coxarum and is irregularly triangular in shape. The ilia and acetabular part of the ischia form the lateral walls of the pelvis.

ii) the ischium; is next in size to the ilium and forms the caudal part of the floor of the pelvis. The caudal lateral portion of the ischium is commonly referred to as the pin bone or tuber ischii. The anterior border of the ischium forms the posterior boundary of the obturator foramen.

iii) the pubis is the smallest of the three bones of the os coxae and forms the cranial portion of the floor of the pelvis. The dorsal or pelvic surface is smooth and usually concave in females. The cranial medial border of the pubic bone provides attachment for pre-pubic tendon while the caudal border forms the cranial boundary for the obturator foramen.

The acetabulum is formed by fusion of the ilium, ischium, and pubis. These bones form a cotyloid cavity in which the head of the femur lodges.

The Ligamentous Pelvis consists of two types of ligaments:

i) The sacro-iliac ligaments are attached to the medial wing of the ilium and the lateral portion of the sacrum and summits of the sacral spines.

ii) The sacro-sciatic ligaments completes the lateral wall of the pelvic cavity behind and between the sacrum and coxae. This ligament extends from the lateral border of the sacrum and the transverse processes of the first two coccygeal vertebrae to the ischiatic spine and tuber ischii.

Functions of the Sacro-Sciatic Ligaments are:

a. They furnish attachments for the vulva and gluteal muscles.

b. They prevent the posterior portion of the pelvis or ischia from rescinding from the sacrum.

c. They form a strong and flexible wall to the pelvic cavity.

d. Under pressure of parturition, they yield sufficiently to allow the constricted pelvic outlet to equal the bony pelvis.

THE OVARIES

The ovary lies inside the abdominal cavity. It performs endocrine (steroidogenesis) and exocrine (ovulation) function. The predominant tissue of ovary is the cortex covered by germinal epithelium.

Ovary is attached to the peritoneum through mesovarium on one end while the other end is free. The ovary is composed of medulla and cortex and is surrounded by germinal epithelium. The medulla consists of irregularly arranged fibroblastic connective tissues and nervous and vascular supply. The cortex contains ovarian follicles and corpora lutea during various stages of estrous cycle.

The primordial germ cells arise extra-gonadally and migrate through the yolk sac mesentery to the genital ridge. During fetal development the oogonia are produced by mitotic multiplication. Oogonia, through meiotic division lead to formation of several million oocytes. The division is arrested in prophase. Up to puberty the number of oocytes is reduced, remaining less than 100,000 at birth.

Arterial blood flow to the ovary alters in proportion to luteal activity. Restriction of blood flow results in premature regression of corpus luteum. Blood flow to the bovine artery is the highest during the luteal phase and lowest during ovulation. Unilateral ovariectomy results in an increase in size of the remaining ovary, called as compensatory hypertrophy.

Functions

The ovary functions as an endocrine as well as an exocrine organ. The endocrine functions of ovaries have been discussed in Chapter 1 of this book. The exocrine functions comprise folliculo-genesis, ovugenesis and ovulation.

Ovarian follicles are fluid filled cavities, lined with estrogen secreting cells. The oocytes are contained inside the follicles and begin their maturation. Follicle stimulating hormones (FSH) regulate the growth of ovarian follicles as a primarily hormone.

The estrogen-secreting-cells line the follicle and surround the egg in response to a rising level of another pituitary hormone, luteinizing hormone (LH) and secrete estrogen. Estrogen levels goes on increasing and during the standing heat reach upto maximum. When the LH levels reach the highest levels (LH surge), it is accompanied by released of egg from the follicle (ovulation).

THE OVIDUCTS

The oviducts connect the ovaries with the uterus and are found as paired elongated tubes. Oviducts are also called as fallopian tubes. Isthmus connects oviducts with the uterine horn and is harder. It continues as the ampulla which is softer in texture and the ampulla leads to infundibulum, a funnel shaped structure. During estrus the infundibulum collects the ovulated egg and guides up to the oviduct and then the isthmus-ampullary junction.

Oviducts are suspended in a peritoneal fold (the mesosalpinx) derived from the lateral layer of the broad ligament. The parts of the oviduct are the isthmus, ampulla, and, the infundibulum with its fimbriae.

The isthmus is the constricted portion lying next to the uterus; this merges with the dilated section known as the ampulla which makes up half the length of the oviduct. The last part of the ampulla is funnel shaped and is called infundibulum.

The opening of the oviduct into the abdominal cavity is called the ostium abdominale, which is surrounded by a fringe of irregular processes called fimbriae, which form the cranial extremity of the tube. The isthmus or caudal end of the oviduct continues directly into the uterine horn at the utero-tubal junction.

In the mare the isthmus opens into the uterine lumen through a small slit on a mound or papilla. In the cow and ewe there is marked flexure at the transition of the isthmus with the elongated curving end of the uterine horn. The latter has a very narrow lumen.

Functions

The oviduct is the site for gametes transportation, fertilization and early cleavage. During estrus transportation of sperm and ovum is caused in opposite direction simultaneously, towards the insthmus-ampullary junction, which is the site of fertilization. After estrus the transportation is directed towards uterus only. Transportation of gametes is facilitated by contraction of oviducts, movements of ciliated epithelial cells and oviductal fluids.

Fertilization takes place in the lower portion of the ampulla following which the fertilized ovum or ova enter the uterus. The oviducts are torturous, wiry and hard, feeling almost cartilaginous when rolled between the fingers. They are difficult to palpate rectally but bimanual vaginal and rectal examination, as well as palpation of the ovarian bursa are of assistance in their evaluation.

THE UTERUS

Uterus is the major component of the reproductive tract, having a muscular-membranous structure. It lies in the abdomen below rectum and comprises of a body and two horns (called as cornua) and a cervix. The uterine horns are attached with the corresponding oviducts. The relative proportions of each, as well as the shape and arrangement of the horns vary from species to species.

In cattle, sheep, and horses, the uterus is of the bipartite type. These animals have a septum that separates the two horns and a prominent uterine body (the horse has the largest). In swine, the uterus is of the bicornuate type. The horns are folded or convoluted and may be as long as four to five feet, while the body of the uterus is short. In human uterus the body is large and the horns do not exist. In rabbits the horn open into vagina separately. In farm animals the uterus is intermediate between the two extremes. Both sides of the uterus are attached with the abdominal wall by broad ligament. Length of reproductive organs in various farm animals is given in Table 2.1.

Table 2.1 Length (cm) of reproductive organs in various farm animals.

Organ	Cow	Ewe	Sow	Mare
Oviduct	25	15-19	15-30	20-30
Uterine horn	35-40	10-12	40-65	15-25
Uterine body	2-4	1-2	5	15-20
Caruncles	70-120	88-96	0	0
Cervix	8-10	4-10	10	7-8
Vagina	25-30	10-14	10-15	20-35
Vestibule	10-12	2.5-3	6-8	10-12

Internal layer(endometrium) of the uterus comprise a mucus membrane, the middle layer comprises smooth muscles (myometrium) and the outer layer is serous in nature. Uterus is an endocrine gland secreting reproductive hormones.

Functions

Endocrinological functions of the uterus have been discussed in Chapter 1. Other uterine functions include reception of the fertilized ovum, for the nutrition and protection of the fetus, and for the initial stage of expulsion of the fetus at the time of parturition. In the domestic animals the endometrium of the uterus is the only structure than can form sufficient placental attachment to result in normal development of the embryo and fetus.

The uterus supports the spermatozoa through transportation inside the oviduct and supports the growing fetus through providing nutrients and the environment for development. At the end of gestation, the muscular wall of the uterus contracts and cause expulsion of the calf. About 40 days postpartum in cattle and buffaloes, the uterus comes to its normal size through involution.

THE CERVIX

The cervix lies caudal to the uterine body. It comprises thick walls, narrow lumen and is a sphincter-like. It is located in the pelvic cavity, the pelvic brim or the abdomen, depending upon its size and contents. Its wall is harder, thicker and more rigid than walls of the uterus or the vagina.

In ruminants the cervix is closed, except the estrus and parturition, and it separates the uterus from outside environment. In cattle the length of the cervix varies from 1.5 to 8.0 cm. Annular rings are the interlocking ridges and complex folding of the lumen mucosa. These rings provide resistance during insertion of an insemination pipette through the cervical lumen.

The cervix remains isolated from the uterus through muscular rings that provides a seal to the pregnant uterus and prevents entry of any material. At the end of gestation the seal is removed and dilation of the cervix leads to expulsion of the fetus.

THE VAGINA

The vagina provides a connection between the cervix and vulva. It is a muscular membranous structure lying in the pelvic cavity dorsal to the bladder. It continues from the cervix and extends up to the urethral orifice. It is separated from the vulva through the hymen, a circular constriction.

The vaginal muscular wall consists of an inner circular layer and an outer longitudinal layer. The muscularis is well supplied with blood vessels, nerve bundles and group of nerve cells. The vagina is lined with a layer of glandular, stratified squamous epithelial cells. In cow a layer of mucus cells lines the inner surface of the vagina. At different stages of the estrous cycle the vaginal secretion vary due the changing levels of the reproductive hormones.

The vagina is capable of dilatation up to a great extent. At copulation the penis is inserted into vagina and semen is ejaculated into it. At the end of gestation the vagina provides birth canal for parturition.

Vagina produces antigens against spermatozoa and bacteria through the plasma cells lying beneath the epithelium. This production increases during the luteal phase. Vaginal fluids are secreted which are composed of secretions from the vaginal wall, vulva, cervix and the uterus.

At copulation, semen is deposited and coagulated in the vagina until spermatozoa are transported through the macromolecules of the cervical mucus column. Most of the seminal plasma is expelled or absorbed in the vagina. pH of the vagina is unfavorable for the spermatozoa which is made favorable by a complex interaction among the vaginal fluid, seminal plasma and cervical mucus leading to formation of a buffering system, providing protection to the spermatozoa.

THE VESTIBULE AND THE VULVA

The vestibule connects the vulva with the vagina at the external urethral orifice. The urethra opens into the cranial ventral portion of the vestibule. The vestibule has several circular or sphincter-like muscles that close the genital canal to the outside.

During parturition the vestibule acts as the point of attachment for the entire genital tract to contract upon when expelling the fetus. The vulva lips normally come together evenly and do not gape, and the vulva opening usually lies at about 90 degree angle to the pelvic floor. The clitoris is about 3 to 6 cm long in the larger animals but is practically hidden in the tissues between the vulva and ischiatic arch. It is the homologue of the penis and in the mare and sow the glans clitoris is well developed and lies in a true fossa.

Vulva is located as the external component of the reproductive tract and comprises the two labia, the dorsal and ventral commissures concealing the clitoris. Labia majora is richly endowed with sebaceous and tubular glands and fat deposits with

the same outer surface structure as the skin. The labia minora has a core of spongy connective tissue.

PUBERTY AND FACTORS AFFECTING PUBERTY

Puberty is manifested by the bulls through exhibition of libido and production of spermatozoa, while the heifer exhibits reproductive cyclicity and occurrence of estrus at 21 days intervals. Heifers do not produce new oocytes while the bull produces spermatozoa continuously. At birth the heifers possess oocytes in maximum number. However, after the onset of puberty, the oocytes undergo development and maturation. It occurs in a wave pattern after commencement of puberty and continues for the rest of life. The waves of development and regression of follicles occur on approximately a weekly basis. The dominant follicle contains a mature egg which is released at end of an estrous cycle at an interval of 21 days in cattle and buffaloes; called as ovulation.

Superovulation is the release of more than one ovum per estrus through administration of exogenous hormones. Superovulation may be induced in younger heifers of up to the age of 2 months through execution of hormonal treatment. Some of the commercial companies produce embryos from younger dairy heifers through hormonal treatment.

Hormonal Changes are seen with the onset of puberty. Before puberty, the required levels of LH and FSH are not available from the pituitary, for supporting for development and maturation of oocytes. The onset of puberty is manifested by release of LH and FSH coupled with and an increasing responsiveness of the ovary and oocytes to these hormones.

Factors that affect puberty may be classified into three categories: the breed, age and nutrition. These factors can enhance the onset of puberty by several months. Other factors may alter the age at puberty (AAP) up to a smaller extent of about a month.

The onset of puberty may be attributed to maturation of the central nervous system which is associated with initiation of release of LH and FSH. The exact mechanism of maturation of the brain to support reproductive activities is still unknown. Genetic merit of the heifer will provide the primary and predominating factor regulating the onset of puberty. Age at puberty varies across the breeds and individuals within the same breed and herd. Feeding management and treatment of the heifers with reproductive hormones can increase or decrease the AAP. Management strategies can result in reduced or delayed age at puberty but this variation may be limited to 1 or 2 months only.

Breed affects the age at puberty. The effect of breed on the age at puberty may vary from 6 to 20 months of age. Heifers may be made to calve at 24 months, if they are bred at an age of 14 or 15 months. If a heifers gets into puberty and manifests 2 to 3 estrous cycles before the commencement of breeding season, the chances of getting pregnant to the first service increase. Age at puberty in crossbred heifers is smaller than purebred heifers. Heritability of reproductive traits is low; however, heterosis of reproductive traits is higher. Maintenance of crossbred heifers in a commercial dairy herd may lead to reduction in age at puberty by 12 to 14 months, and improvement in reproductive efficiency.

Nutritional status affects the onset of puberty in farm animals. For maintaining successful reproductive processes in replacement heifers, an optimum feeding regime is required. Optimum nutritional status accelerates the onset of puberty by few months by under-nutrition results in retarded growth of the body system and impedes body weight gain leading to delayed puberty for a much longer time. The heifers must reach a critical weight during commencement of the breeding season, if they are to be bred successfully. Under nutrition prevents secretion of LH and FSH in a different way than age or genetics. An underweight heifer attaining sufficient age may show a rapid weight gain through higher nutritional plan and will manifest onset of puberty.

Weight at puberty is more critical than age. After reaching a certain weight (about 70% of the adult body weight) the heifer will exhibit the symptoms of puberty. This critical weight may be brought about at an earlier stage of life through appropriate feeding strategy.

The feeding plan for replacement heifers should include good quality forage or grazing as well as ingredients of high energy contents. The scale of addition of supplements should range from 0.5 to 1% of body weight. In addition to energy, the availability of protein may also be ensured for an earlier onset of puberty. In a research study, 40% of the heifers fed a low protein (9%) diet showed symptoms of puberty compared to 90% heifers fed adequate nutrition. The study further revealed that addition of extra energy (i.e. corn) in the heifer diet could not overcome the effect of protein deficiency. Similarly, providing extra protein did not compensate the deficiency of energy availability. The growing heifers required crude protein at a level of 11 and 12%. Vitamins and minerals are required for proper reproductive health. Deficiencies in vitamin A, phosphorus, copper and zinc may lead to delayed puberty and lower conception rate. Before the onset of normal breeding season, the spring born heifers must manifest the signs of puberty. Weight of the heifers may be recorded at appropriate intervals to monitor the growth rate and meet the target weight before the onset of breeding season. The slow growing heifers need improvement in the feeding plan to get the desired weight before commencement of the breeding season.

BREEDING SEASONS IN FARM ANIMALS

It is generally accepted that livestock productivity and reproductive efficiency in the temperate regions is higher than the tropical ones. A major factor associated with the low reproductive performance, is seasonal influence through poor forage availability and thermal stress in the harsh, hot and often semi-arid or wild environment.

Reproductive activities occur in farm animals throughout the year. However, during certain seasons of the year the seasonal stress cause suppression of reproductive activities. Consequently, the occurrence of estrus and conception rate, are reduced. Hence certain seasons may be normal breeding season (NBS) and other low breeding seasons (LBS) for certain species. A brief review of seasonality of reproduction in some domestic livestock species follows.

BUFFALOES

Studies at Peshawar have shown an opposite breeding season in cattle and buffaloes (20). Spring was found as a normal breeding season for cows and low breeding season for buffaloes. This was based on occurrence of estrus, inseminations and higher rates of pregnancy. The trend in autumn was opposite for the two species.

Our group studied seasonality of reproduction in Nili-Ravi dairy buffaloes (18, 19). Autumn and winter were found as NBS and spring and summer as LBS. NBS calving buffaloes showed shorter postpartum estrus interval of 56 days while the interval was longer (91 days) in LBS calving buffaloes. Postpartum milk progesterone levels were lower during LBS than NBS. In spite of the higher incidence of estrus events (32%) and postpartum uterine involution interval (33%) during summer, the lower progesterone levels were attributed to summer infertility in buffaloes. Post-partum ovulation interval was shortest during autumn.

The factors contributing to seasonality of reproduction were classified as nutritional and non-nutritional factors. Energy intake was found as the highest during NBS contributed by the non-leguminous fodders like maize and sorghum. Better reproductive performance during NBS was associated with low intake of protein. The crude protein-energy ratio was also lowest during the NBS. The higher ratio of protein during NBS was not favorable for reproductive performance because the protein was ruminally degradable and hydrolyzed into ammonia and than converted into urea in the liver. Resultantly the milk production is depressed and fertility.

Milk progesterone levels showed an opposite pattern to the ambient temperature. Minimum ambient temperature started rising from 20 °C in February to 46 °C in June and decreased onwards. The change in milk progesterone levels was just opposite. During start of the autumn the decreasing daylight and ambient temperature coincided with the commencement of breeding season.

GOATS

In goats breeding starts in summer in the countries where the change in the duration of the daylight is large. In the tropical countries with little change in the daylight length, the kidding is observed throughout the year. Fertility in goats in reduced during certain month's differencing from region to region. This reduction in fertility was attributed to the temperature regime and feed availability. It is a common observation in the tropics that estrus occurs shortly after the rainy season or even after sporadic rains in dry seasons(4)

SHEEP

Sheep show seasonal anestrus which is associated with the basal levels of progesterone due to absence of pre-ovulatory gonadotropin surge and ovulation. It has been suggested that seasonal anestrus may be due to a reduction in the positive feedback sensitivity of the hypothalamus-pituitary axis since it has been shown by some workers that there is seasonal variation in the response to exogenous estrogens. The ovaries of anestrous ewes are not totally inactive. Indeed periods of early follicular growth and regression occur through out the inactive period. However, the frequency of episodic LH secretion was lower.

PHYSIOLOGY OF ESTROUS CYCLE

Estrous Cycle is the period from one estrus to the next. It is also called as sexual cycle or reproductive cycle and comprises changes in the physiological and behavioural status of the animal (Figure 2.2). Estrous cycle continues constantly after the onset of puberty in the female animals. It enables a female for getting pregnant and repeat at a 21 days interval in cattle and buffaloes.

The ovarian follicles develop in a wave-like pattern during the estrous cycle. This development is supported by tonic levels of FSH from the pituitary gland. The dominant follicle ovulates and the site of ovulation gives rise to formation of corpus luteum. The corpus luteum prevents development of more follicles from maturation and causes their atresia through negative feedback. The interval between two consecutive estrous cycles is known as the length of the estrous cycle.

Phases Of Estrous Cycle are two: the pro-estrus and estrus constitute the estrogenic phase and the metestrus and diestrus constitute the progestestational phase. Estrogen remains dominant during 4 days of the estrous cycle, and progesterone remains dominants for the remaining period. A sound understanding of the reproductive cycle of an animal enables a veterinary practitioner to introduce appropriate management practices for maintaining reproductive efficiency at its maximum level.

PROESTRUS

Proestrus is the first part of the estrus. It occurs 2 to 3 days before the onset of estrus in cattle and comprises growth of ovarian follicles and production of estrogen. The higher estrogen levels lead to enhancement of blood supply to the reproductive system and increasing tone of the uterus. The cervix and vagina get stimulated for production of higher levels of estrogens accompanied by secretion of mucus and discharge per vagina.

ESTRUS

During estrus there is a higher level of sexual activity and receptivity for breeding. The animal in estrous exhibits mounting other cows, standing for mounting by others; the vulva gets swelled; there is restlessness, discharge of clear vaginal mucus and bellowing. These are called the primary behavioral signs of estrus in cows. The estrus gets repeated after every 21 days and lasts approximately 18 hours. In cattle the time of ovulation is about 12 hours after the standing estrus ends.

The cause of behavioral signs of estrus is the higher levels of estrogens secreted by the grafian follicles and acting on the central nervous system. This causes the cow to become restless, start bellowing, loses her appetite, and shows a decline in milk yield. The higher levels of estrogen increase the blood supply to the reproductive tract leading to increased glandular secretion and producing clear viscid mucus. The mucus is discharged per vulva and may be observed by the attendant. The signs of behavioral estrus remain for 14 to 18 hours.

The period of sexual receptivity in cow is shorter, when she allows mounting by a bull or other cows. Ovulation does not occur until 12 to 16 hours of the end of estrus. The shorter period of standing estrus necessitates proper estrus detection strategy, so that no estrus could be missed.

METESTRUS

Metestrus follows the estrus, immediately. Sexual receptivity and activity are the characteristics of this period. Ovulation occurs at the end of estrus. Ovulation occurs during metestrus, in response to LH surge. Rupture of the grafian follicle releases ovum, called as ovulation. The follicular cavity is filled by blood and luteal cells and corpus luteum (CL) is developed. Duration of the metestrus is about 2 to 3 days. The infundibulum of the oviduct collects the ovum which starts transportation toward the uterus. After reaching isthmus-ampullary junction in the oviduct, the ovum encounters with spermatozoa and fertilization occurs. So, it is know as the site of fertilization.

During estrus, there is an engorgement of the reproductive tract with blood supply. It is followed by a discharge of blood stained mucus during metestrus. This is called as postestrus bleeding. The postestrus bleeding indicates that estrus has occurred one or two days earlier. Post-estrus bleeding is the result of estrogen withdrawal. It is different from the menstrual bleeding in primates which occurs due to progesterone withdrawal.

DIESTRUS

Diestrus is the part of the estrus cycle when the corpus luteum (CL) is present and is functioning. It constitutes the major portion of the estrous cycle. Formation of corpus luteum occurs at the ovulation site. The CL will be formed as an organ for producing progesterone which is called as the luteal phase of the estrous cycle. Formation of CL is independent of pregnancy establishment. If pregnancy occurs, the CL will continue up to parturition while in case of no pregnancy the CL will regress on day 17 of the ovulation and the estrous cycle will be repeated.

Various phases of estrous cycle will occur under the control of hypothalamus and pituitary gland in a healthy state of the animal, during normal breeding season and under appropriate management environment. If anything abnormal happens, the estrous cycle will cease to occur. In addition, for improved reproductive efficiency, the estrous cycle may be intervened at any time.

STANDING ESTRUS AND OVULATION

Standing estrus is the phase of estrous cycle when the female is sexually receptive for breeding. It is the most vital sign of the estrous cycle and is also known as standing heat. Duration of estrus in cattle, on an average is about 15 hours ranging from 6 to 24 hours. During standing estrus in cattle, a female stands for mounting and allows mounting by other animals. Symptoms of the standing estrous progress gradually. Before the standing estrus the cow mounts on other animals; and becomes nervous and restless. After the onset of standing estrus, the cow shows roughed up tail-head; there is a discharge of clear mucus from the vagina, and the vulva becomes swollen. The cow stands for mounting by other animals and this confirms that the cow is in standing estrus.

During the standing estrus the mature grafian follicle secretes estrogen that exerts a positive feedback on pituitary gland and cause LH surge. It leads to rupture of the grafian follicle and release of ovum, called as ovulation. Ovulation occurs 24 to 32 hours after the onset of standing estrus. The released ovum will be guided towards the oviduct and will be transported towards the isthmus-ampullary junction, which is the site of fertilization. After ovulation, corpus luteum will be developed at the site of fertilization and another estrous cycle will be initiated. Duration of the estrous cycle in cows is 21 which may increase or decrease by three days.

THE CORPUS LUTEUM

After ovulation the estradiol secreting follicular cells are converted into progesterone secreting luteal cells that form the corpus luteum (CL). Corpus luteum develops at the site of ovulation. The CL remains the dominant structure on the ovaries during the luteal phase of estrous cycle which is the longest phase.

The CL secretes progesterone, which controls various physiological functions in the reproductive system. The uterus is prepared for pregnancy, and after fertilization pregnancy is continued till parturition. The higher levels of progesterone during pregnancy exert a negative feedback on the hypothalamus and pituitary glands which suppresses the maturation of grafian follicle and ovulation.

At the beginning of estrous cycle the CL develops and increases in size with a parallel increase in progesterone production also increases. The higher levels of progesterone can be detected 5 days after standing estrus. In case of no pregnancy the progesterone concentrations will start decreasing around day 17 of the estrous cycle. After ovulation the estrous cycle will repeat again.

FOLLICULAR WAVES

Follicles development continues throughout life of a mammal in wave-like patterns, and these follicular waves may be observed during most reproductive stages. The waves are supported by the tonic center of the hypothalamus and continue during the pre-pubertal period, the estrous cycles, pregnancy, and also during the postpartum anestrous period.

Following each ovulation, a new follicular wave is initiated. Recruitment of many follicle s occurs out of a pool of smaller follicles present on the ovary and start growing in the form of follicular wave. The recruited follicles continue growing and reach the stage

of antral follicles. One of the follicles is selected for maturation as a grafian follicle and grows as dominant follicle. The dominant follicle secretes inhibin which suppresses production of FSH and prevention of development of other antral follicles.

The dominant follicle develops in the absence of progesterone, and the levels of estradiol increase. It is followed by LH surge which causes rupture of follicle and ovulation. In the absence of LH surge, there will be no ovulation from the dominant follicle; it will be followed by atresia, and initiation of a new follicular wave.

THE ANESTROUS PERIOD

Anoestrus is the period of cessation of estrous cycle. This is observed in heifers during the pre-pubertal life and in cows during the postpartum period. Folliculogenesis continues during the anestrous period, but maturation of follicles to the status of grafian follicles and ovulation do not occur. During anestrous period the estrous symptoms and standing estrous are absent, so breeding and occurrence of pregnancy do not occur.

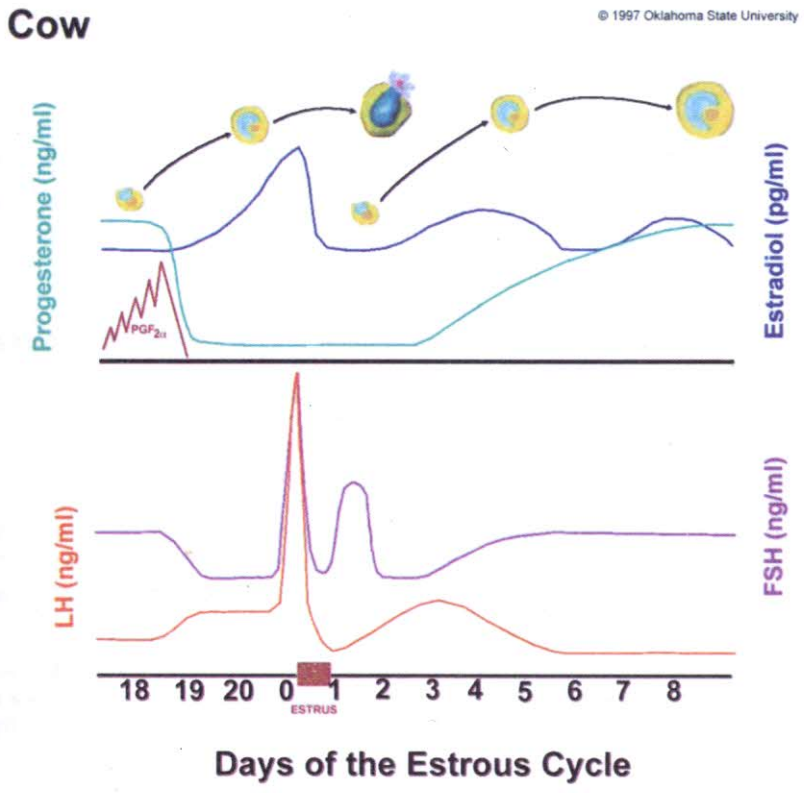


Fig. 2.2 The estrous cycle of cow (Geisert,1999)

SEXUAL BEHAVIOUR

Behavior possesses an important role in mammalian reproduction. It is essential for mating and suckling of young ones, which have been made attractive by nature for survival of the mammalian species. Behavior of domestic animals has been influenced by domestication and husbandry practices like confinement in paddocks, yards or indoor pens, segregation of sexes, controlled breeding, caesarian delivery, enforced weaning, imposed proximity with other animals and the presence of man.

Male and female gametes are to be brought together for the purpose of fertilization. Various patterns of courtship, motor activities, display and postures are exhibited to cause an attraction between male and female leading to copulation. The coordination of motor pattern leading to insemination of the females has been achieved by the evolution of an orderly series of responses to specific stimuli.

COMPONENTS OF SEXUAL BEHAVIOR

The first step in reproductive behavior is the encounter with the sexual partner. Under wild environment the reproductive behavior is influenced by the pre-existing social infrastructure and the home-range behavior of males and females. In the roe deer and muntjak antelope, males and females live in a limited area, the boundaries of which are defended against any intruder of the same sex. The territories of males and females are overlapping, with permanent association between potential sexual partners.

The basic unit in farm animals under feral conditions is matriarchal, consisting of a female, her adult female offsprings and their immature young. Such a matriarchal herd is remarkably stable. It persists after a temporary dispersion of its members or mixing in large groups of several hundred individuals (African antelopes, bisons). Experimentally, cows reared together from birth form such a stable group even in the middle of a large herd. Such a bond limited to the dams and their female offsprings, could be the basis of the social organization in ungulates.

Copulation is prerequisite for insemination, fertilization and gestation. The components of copulatory pattern are sexual arousal, courtship, erection of penis, protrusion of penis, mounting on the female, intromission into the vagina, ejaculation, mounting down and refractoriness. The duration of courtship and copulation are longer in horses and swines and shorter in cattle and ship.

COURTSHIP

Courtship is simpler in mammals than birds, fish or arthropods. The management of farm animals facilitates the contact of males and receptive females. Even in the wild, the pattern of courtship in mammals is simple, involving only a limited number of ritualized displays.

In male, sniffing and licking the females are the most frequent patterns, suggesting an important function of chemical communication through olfaction. The males of domestic animals smell the urine of the female and raise his head with lips curled, in the ritualized "Flehmen" reaction.

In females an increased motor activity is observed after commencement of estrus, manifested by restlessness and movement at slightest disturbance. Receptive cows and goats exhibit increased frequency of non-specific bellowing, mounting, standing to be mounted by other females. The males and females sniff each other's perineal region and move in a circle. Estrous mare tends to urinate frequently in the presence of stallion. After approaching and stimulation by the male, the female assumes a mating posture.

The bull detects pro-estrus in cows 2 days before the estrus and remains in her general vicinity. This is called "guarding" of the female. The body axes are parallel and body orientation is head to tail.

MATING

It is the terminal phase of the courtship and is manifested by immobile standing of female, mounting of female and ejaculation. During the pro-estrus the male mounts on the female several times and the erection takes place partially. This mounting is unsuccessful. The bulls excrete "dribblings" of accessory fluid derived from the Cowper's gland. It is different from the seminal plasma emitted by the seminal vesicle during ejaculation.

If the female is receptive, the copulation may occur rapidly. The male rests his chin on the female, and she in returns responds by standing. The male than mounts, fixes his forelegs on the female, grasps her firmly and performs rhythmic pelvic thrusts. Duration of copulations varies widely among species. Bulls and rams ejaculate instantaneously on the first intromission. Boars maintain intromission for as long as 20 minutes with a single ejaculation. The stallion oscillates the pelvis several times resulting in engorgement of the penis with blood and making it rigid for maximum intromission. In rodents a series of mounting and intromission take place.

ENDOCRINE MECHANISM

In the male androgens secretion takes place in several peaks within 24 hours, reflecting the pulsatile release of pituitary gonadotrophins. Stallion produces large quantities of estrogens compared to other males. Testosterone support libido and ejaculation. In the female estrogens are produced only during a few days of the estrous cycle. An appropriate endocrine balance is necessary for maintenance of female sexual behaviour.

ESTRUS DETECTION BY MALE

A female becomes receptive during the standing estrus. A male approaches the female and detects estrus during the course of courtship sequence. The female posture and motor reaction to the testing male is essential for the engagement. The final clue to the estrus detection is the immobility of the female for mounting. The mating posture is often adopted spontaneously when a female is approached by the male or by another female.

CAPTIVE BREEDING

Captive breeding provides a feasible system for *ex situ* conservation of wild animals. Welfare of animals influences their reproductive performance. It has been reported that reproductive activities are suppressed by stress however mild stress has been found to favor reproductive efficiency in some species. Good welfare of captive animals is associated with successful breeding, although an opinion exists that productivity alone reflects welfare may be false.

It has been a common belief among the zoo community that the breeding can not become successful without a good welfare system, however it has yet to be proved. Sufficient scientific information are lacking regarding the success of captive breeding program, because of the very slow nature of this process. However, extensive investigations are required under captive and free environment to document the success of various breeding protocols.

FOLLICULOGENESIS

Ovarian follicles are the compartments of ovary which enable the ovary to fulfill the dual functions of steroidogenesis and gametogenesis. Ovarian follicles are found on the surface of ovary in different stages of development. They contain the oocytes in alive and viable state.

Follicular development occurs through various stages comprising primordial follicles, primary and secondary follicles, tertiary follicles and Graafian follicles. Follicles develop under the influence of FSH and ovulate under the influence of LH hormones. Follicular development complete within 20 to 180 days in farm animals.

PRIMORDIAL AND PRIMARY FOLLICLES

Primordial follicles are present in mammalian females which are produced during prenatal or immediate postnatal periods. A primordial follicle consists of a diplotene primary oocyte surrounded by a single layer of granulosa cells. They form the largest number in the ovary.

Some of the primordial follicles grow continuously throughout the life or at least the reserve is exhausted. When the primordial follicle is released form the reserve, it continues to grow until ovulation or atresia (degeneration). During early neonatal life, the primordial follicles become localized in the peripheral cortical zone beneath the tunica albuginea.

Primordial follicles develop into ***primary follicle*** with increase in number of granulosa cells through mitosis. The cell wall becomes cuboidal. It has been found that follicular growth initiates in a centrifugal pattern inside the ovary. Activation of primordial follicle is associated with a change in the shape of granulosa cells from flattened to cuboidal ones, during their propagation.

ANTRAL FOLLICLES

With the accumulation of follicular fluid in ***antrum*** within epithelial cells, the primary follicles develop into a***ntral follicles***. These may also be called as secondary or

vesicular follicles. In bovine ovaries the follicles appear in ovaries with the order: primordial 90 days, primary 140 days and antral follicles 210 days. In sheep these intervals have been found to be 75, 100 and 120 days. Antral follicles are present on the surface of ovaries throughout the estrous cycle. One fully-grown-oocyte is present in each one of the antral follicles.

The oocytes may be aspirated from antral follicle through a syringe into a Petri dish and examined under an inverted microscope with a magnification of 10x to 15x. The oocytes are attached with a mass of granulose cells known as cumulus oophorus. The cell surrounding and in immediate contact with the potential ovum are called as corona radiata. The oocytes so collected may be utilized for in vitro maturation and fertilization which may be further used for research purpose or transfer to the recipients.

GRAAFIAN FOLLICLES

The antral follicles grow to form the Graafian follicles which are the terminal phase of the follicular growth. Graafian follicle secretes estrogen which is associated with estrous symptoms. The Graafian follicle acts as dominant follicle which suppresses development of further follicles on the ovary through secretion of inhibin.

The Graafian follicles rupture under the influence of luteinizing hormones causing ovulation. The inner wall of the follicle develops into macro and microscopic folds that penetrate the central cavity. The granulosa cells degenerate and luteinize to form lutein cells of the corpus luteum.

Granulosa cells of the follicles assist in maturation of the oocytes. Ovaries secrete inhibin which through granulosa cells of the larger antral follicles, suppress FSH secretion. Estrogen is produced by the Graafian follicles which cause estrous symptoms. Estrogen secretion decreases rapidly at the time of the LH peak. LH causes ovulation through rupture of the follicle.

FOLLICULAR GROWTH

Primordial follicles are activated to form primary follicles and then by developing an antrum they become antral follicles. Various stages of development include basal growth leading to recruitment, followed be dominancy growing to the preovulatory stage. Fig. 2.3 shows follicle and oocyte development in cattle. Gonadotrophins regulate the recruitment and further development. The antral follicles produce estradiol that enables them to survive. With the decreasing levels of FSH the selection of antral follicles occur. Later on their sensitivity to the FSH increases and then they develop further under the higher concentrations of LH. The antral follicles must respond to the gonadotropin stimulation which enables them to survive and develop to advanced stages.

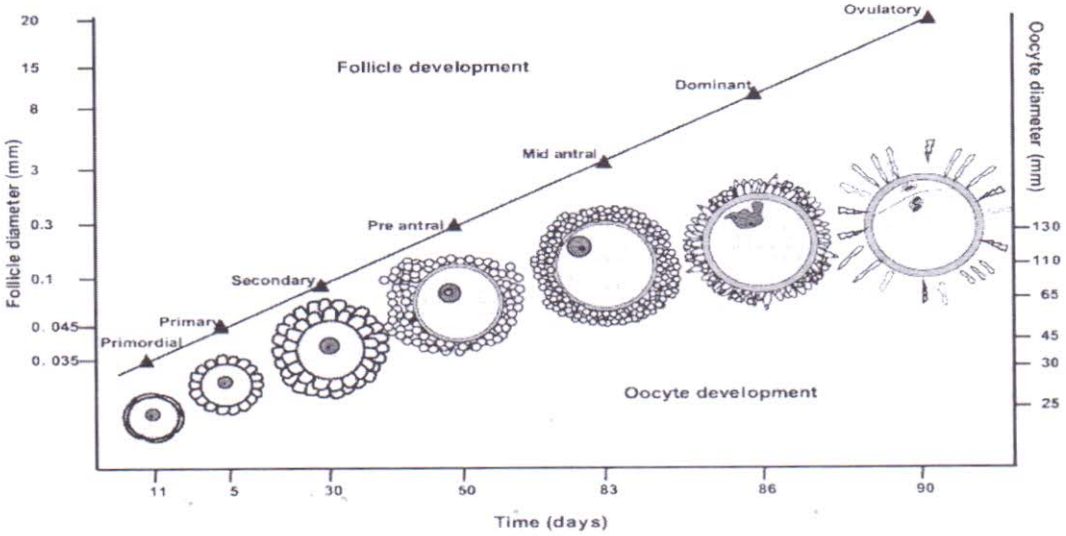


Fig. 2.3 Development follicles and oocytes in cattle. (The growing oocytes in the follicles are supported by the follicular or granulosa cells. Relative diameters of follicles, oocytes and duration of various stages of follicle development (Fair 2003).

FACTORS AFFECTING FOLLICULAR GROWTH

Granulosa cell get proliferated under the influence of various factors and the factors are also considered favorable for activation of primordial follicles. Studies have concluded that bone morphogenic protein (BMP-7) stimulate granulosa cell division which, in turn, promote stimulation of primordial follicles. Another regulating factor has been identified as IGF-I, responsible for primordial follicles stimulation in addition to stimulation, proliferation and differentiation of graulosa cells in swines, sheep, rats and human beings.

Sensitivity of the growing follicles is affected by the Anti-mullerian inhibiting hormone (AMH), which seems to regulate the activation of primordial follicles. This regulation is based upon inhibition of selection of primordial follicles into the growing pools.

The role of several other regulating factors have been investigated including kit ligand, bone morphogenetic proteins (BMP-15), basic fibroblast growth factor (bFGF), and leukemia inhibitory factor (LIF). Some local factors have also been identified within the ovary, which act locally in stimulating the development of primordial into primary follicle.

Recent studies suggest that the oocyte provides the driving force for its own development, occurring after the stage of the primary follicle. The primary action of growth and differentiation factor-9 (GDF-9) is to develop primary follicles to the preantral stages, conducive for stimulation of the primordial follicles. It leads to an increase in the number of follicles to develop from primordial to primary stage. The granulosa cells produce Steel factor that helps promoting the growth of oocytes up to the size which is species-specific. Then the oocytes secrete a factor called GDF-9 which prevents the

expression of kit ligand in cumulus cells, resulting in sluggish growth and atresia of oocytes.

OOGENESIS

Oogenesis is the formation of ovum taking place in the ovary. Predecessor of ovum are oocytes which are present in the primordial follicles in the ovary. Maximum number of primordial follicles is present in the ovary at birth which is formed during prenatal or immediate postnatal periods. These follicles develop and either ovulate or become atretic and are lost as presented in table 2.2. Developmental stages of oocyte are given in table 2.3. Fig. 2.4 shows graphic presentation of oogenesis in mammals.

OOCYTE FORMATION

Maturation of oocyte takes place in two stages: i) the period of growth and; ii) the period of final nuclear and cytoplasmic preparation prerequisite to fertilization and normal development. When the primordial follicle is released from the reserve, the oocyte and its follicle begin to grow. Oocyte growth is almost complete at the time of antrum formation. The inner cumulus cells actively cooperate to achieve oocyte growth as they establish an active contact with the oocyte cell membrane.

Synthesis of oocyte is completed in the following steps: a) formation of (PGC) primordial germ cells; b) movement of PGC to the embryonic gonadal ridge; c) PGCs get colonized inside the embryonic gonads; d) the PGCs get differented into oogonia; d) propagation of oogonia; e) meiosis commences; and f) meiosis is arrested at prophase-I of meiosis at the stage of diplotene.

The undifferentiated epiblast cells undergo gastrulation and involute to form the primordial germ cells. Embryonic cells change their position through movements called gastrulation, occurring in different modes. The embryonic cells re-arrange themselves into two cells layers is a cell movement that can occur in a number of ways but invariably results in an embryo with two cell layers formed from one.

The primordial germ cells move into the endoderm of the yolk sac. These cells undergo proliferation and migration into the yolk sac and endoderm of the hindgut from where they move towards the gonadal ridges.

Gastrulation may be made visible through the Oct-4 which is a green fluorescent nuclear transcription factor. This factor gets expressed in epiblast cells which are converted into primordial germ cells. When gastrulation completes, the Oct-4 factor gets restricted to PGCs and oocytes. This factor is only detectable the living embryos. Confocal laser microscopy may be used to observation movements of the cells in vivo. Alkaline phosphatase staining may be used in this technique.

The primordial germ cells migrate and reach the gonadal ridge. These cells differentiate under the influence of the sex chromosomes, X-chromosome leading to oogonia and Y-chromosome leading to formation of spermatogonia. These resulting gametocytes propagate through mitotic cells divisions with a high transcriptional activity. The oogonia remain connected among themselves through establishment of cytoplasmic bridges.

The oogonia undergo meiosis and divide into primary oocytes passing through various stages of cell division. Meiosis initiates with leptotene following by zygotene and pachytene stages of meiotic prophase-I. Then it converts into diplotene where the cells division becomes arrested. During the early meiotic stages the cells need, repair proteins for DNA and other factors to support chromosome arrangements and reshaping. This is a complex proper and most of the oocytes undergo regression.

Oogonia develop into oocyte and increase in size during the diplotene-stage and the cytoplasmic organelles increase in number. Meiosis gets arrested at this stage until a follicle becomes atretic or a it matures leading to LH-surge, resuming meiosis and expulsion of first polar body, containing half number of chromosomes, during ovulation. It is followed by ovulation and fertilization, which is associated with second meiosis, expelling the second polar body. But this time only the surplus genetic material is expelled.

OOCYTE GROWTH AND FORMATION OF ZP

The oocytes grow intensively and develop into the pre-antral follicle. The follicular or granulosa cells surround the oocytes and proliferate followed by development of a theca layer around the granulosa cells originating from the interstitial stroma cells. The follicle develops and increases in size up to 150 µm in cow, 200 µm in humans and 300 µm in sow while the diameter of occytes in these species is 60, 80 and 90 µm, respectively.

Growth is essential for getting competency which leads to maturation of nucleus, completing the process of fertilization following by cleavage. The oocyte increase in volume and undergo differentiation. The oocytes undergo a cytoplasmic re-organization under the influence of gene products and organelles. The growing oocytes show an increase in mitochondria, ribosomes, etc. The synthesis of RNA and protein also increase enormously during the period of the occyte growth.

Zona pellucida (ZP) is produced during the growth phase of the oocyte and it comprise a glycoprotein membrane. ZP serves as a protective coat surrounding the oocyte. It comprises of various layers of glycoproteins known as ZP1, ZP2 and ZP3. ZP2 is present at the stage of primordial follicles while the other two layers are secreted during the growth phase.

The zona pellucida contain proteins which support the normal development of follicles. The granulosa cells communicate with each other and with the surface of the ovum (oolemma) through establishment of gap junctions. The granulosa cells communicate with the ovum through gap junctions allowing transfer of various factors. These factors include various types of nutrients and metabolic precursors like nucleotides and aminoacids. Other factors include informational molecules like growth factors, hormones and neurotropins. Meiotic signals (both and inhibitory and stimulatory) are also passed on to the oocytes through gap junctions.

Table 2.2 Oocyte loss during early meiosis

Species	Maximal oocyte number	Oocyte number at birth	Loss of oocytes (%)
Rodents	75,000	15,000	80
Ewes	900,000	82,000	91
Sows	1,200,000	500,000	58
Cows	2,700,000	135,000	95
Women	7,000,000	700,000	90

Hurk and Zhao, 2005.

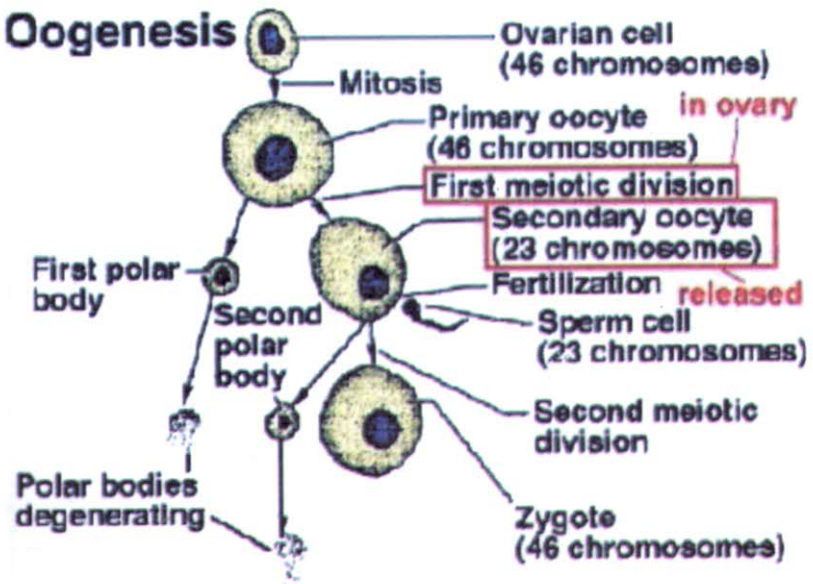


Fig. 2.4 Oogenesis in mammals

Source: Anatomy and Physiology I & II. Springfield Technical Community College Massachusetts. http://distance.stcc.edu/aandp/

Table 2.3 Oocyte development in mammals

Developmental event	Stage of germ cell
Multiplication by mitosis	Epiblast cells → Primordial germ cell
Migration to genital ridge and differentiation according to sex chromosome X or Y guided by stem cell factor	Oogonia
Birth - rabbit, ferret, mink, vole, hamster	
Final inter-phase DNA synthesis Meiotic prophase begin	Primary oocyte
Birth (most mammals)	
Growth of oocyte and follicle	
Puberty	
Follicle maturation	
Ovulation (dog and fox) First meiotic division appear Sperm penetration - dog and fox First polar body emitted	Secondary oocyte
Ovulation – most mammals	
Sperm penetration – most mammals Second meiotic division Fertilization and emission of second Polar body	Pronucleate egg (ootid)

Hafez 1987 (modified)

OVULATION

Ovulation is the release of ovum from the Graafian follicle after it gets ruptured. The Graafian follicle ruptures under the influence of LH surge. The changes occurring during ovulatory process are: i) cytoplasmic and nuclear maturation of oocyte; ii) disruption of the cumulus oophorus cells cohesiveness among the cells of the granulose layers and; iii) thinning and rupture of the follicular wall.

Ovulation may occur alternatively at the right or left ovary like in whales. In cattle, sheep and horses, if one ovulation occurs at right side, the next ovulation may occur at right or left side, independent of the previous one. In rhesus monkey the corpus luteum from the present reproductive cycle suppress the subsequent follicular growth on the same side. It leads to change of ovulation between the ovaries on the two sides, leading to a shorter menstrual cycle. On the other hand, if ovulation occurs on the same side, the duration of cycle is longer.

During the course of development the Graafian follicle begins to protrude from the surface of ovary. Vascularity of the follicle increase except at the centre which is the future point of rupture.

MECHANISM OF OVULATION

i) ***Neuroendocrine:*** Structures within preoptic and suprachias-matic hypothalamus regulate ovulatory discharge of gonadotrophins, which exert cyclic control of ovulation. The tonic center is under negative feed back control of estrogen.

ii) ***Endocrine:*** Lower LH levels are available during the estrous cycle and an LH surge is observed immediately prior to ovulation. LH : FSH ratio regulates follicular development. Two follicular waves are found during an estrous cycle under influence of changes in estrogen and LH levels. Granulosa cells secrete progesterone into follicular fluid which is converted into estrogen by the theca cells. Higher estrogen levels make the pituitary more sensitive to GnRH, which releases LH in larger quantity known as LH surge.

iii) ***Prostaglandins:*** Theca cells of follicle produce prostaglandins which is associated with rupture of follicle. Exogeneous LH increases prostaglandin E and F within the whole ovaries, Graafian follicles and follicular fluid.

OVULATION IN VARIOUS MAMMALS

The onset of puberty may be caused in heifers by the mere presence of an intact or vasectomised bull in a cattle herd. It may also lead to resumption of postpartum ovarian activity in the postpartum cows. The effect of the presence of male on resumption of ovarian activities and induction of estrus has been coined as bio-stimulation in mares. The is facilitated by genital stimulation, secretion of pheromones and other mechanisms.

Induction of behavioral estrus and commencement of ovrian activities have been investigated through use of teaser bull in suckling beef cows. It has been suggested that

the teaser bull increased the induction of ovulation and estrus in cows than the control (ovulation 73.6 vs 40.4%; heat 67.9 vs 32.7%).

Ewes may be induced to ovulation with the introduction of ram. Anestrous ewes get synchronized for estrus with the introduction of ram. The LH hormones show peaks before induction of estrus in these ewes. Efficacy of the urine or wax from the eyes and flanks or shorn wool of the ram was studied for induction of estrus in ewes at the start of breeding season. The study revealed that these techniques are equally effective like the contact with ram, regarding induction of estrus in the ewes.

In marmoset monkeys ovulation is blocked by competitiveness for dominance by the females. The mechanism of blockage has been found to be the pheromonal cues which depress the gonadotrophins production in primates.

In swines the presence of males during the process of insemination enhance the transportation of gametes in the female reproductive tract and as well as the process of ovulation. It has been suggested that during lactation the presence of boar increases the concentration of pituitary FSH the increased concentrations of FSH during the weaning time stimulate the induction of estrus and ovulation.

FERTILIZATION

Male and female gametes are required for mammalian reproduction to produce the young one. The male gametes or spermatozoa are deposited in the female reproductive tract during copulation or artificial insemination. The spermatozoa travel towards the oviduct and the ovum after release from grafian follicle at the ovary, moves downward toward the site of fertilization in the oviduct. The ovum meet with the sperms in the oviduct and this union of the two gametes is called as fertilization. Fertilization gives rise to formation of the zygote (fertilized ovum).

The fertile life of sperm in the female reproductive tract after insemination, relates to the estrous period in female. For example in horse and swine the sperm has got a greater longevity than sheep and cattle. Longevity of sperm life in the reproductive tract ensures increase the probability of viable sperm being present at ovulation when insemination occurs well in advance of ovulation.

During fertilization the spermatozoon and ovum with haploid number of chromosomes fuse to form a zygote with diploid number of chromosomes. The sperm fertilizing the ovum is selected at random out of millions in artificial insemination or billions in case of natural service. In farm animals the number of spermatozoa deposited in vagina is in millions, the number reaching the upper end of the uterus is in thousands, and those reaching the upper end of the oviduct, where fertilization takes place, are in hundreds. Only one sperm fertilizes the ovum for production of a young one.

The mammalian ovum is surrounded by several layers of granulosa cells called as cumulus cells. The spermatozoon penetrates through the cumulus cells and reaches the ovum surface, the zona pellucida. After contact of spermatozoa with the ovum surface, the acrosome reaction initiates on the sperm head. This is associated with release of hyaluronidase and other enzymes facilitating the transportation of spermatozoon. The ovum surface contains receiptor proteins for attachment of

spermatozoa. A series of biological reaction occur facilitating the fusion of sperm and ovum membranes.

THE PROCESS OF FERTILIZATION

The process of fertilization completes in three steps: i) penetration of sperm through the cumulus cells; ii) sperm attachment with the ovum and its penetration through the zona pellucida and; iii) fusion of the sperm and ovum plasma membranes. Peristaltic contraction of the ampulla facilitates the sperm – ovum encounter. Penetration of cumulus oophorus is facilitated by the hyaluronidase present on the acrosome of the bull sperm. In rabbit the cumulus oophorus secrete a substance which may stimulate sperm motility. Figure 2.5 show a sperm attempting to fertilize an egg.

The sperm head attaches with the zona pellucida at specific receptor sites. It facilitates the transfer of haploid genetic materials into the cytoplasm of ovum. After attachment of the sperm with the ovum, the sperm provides an activating factor, which is a protein and it support the process of fertilization and initiation of cleavage. Receptor proteins are present on the surface of sperm as well as ovum. Treatment of sperm with anti-sperm antibody or treatment of zona pellucida with anti zona pellucida antibodies prevents fertilization. These treatments block or mask the receptor sites on sperm or zona pellucida surfaces. These receptors are species-specific allowing attachment of sperm with ovum of the same species.

After attachment of sperm head with the ovum, the sperm enters the zona pellucida within 5 to 15 minutes. Acrosome reaction occurs after attachment of sperm head with the glycoprotein receptors at the zona pellucida. Acrosome reaction results in production of zona lysine which enable the sperm head to digest its pathway through the zona pellucida to the vitelline membrane. The acrosome contains various enzymes such as hyaluronidase, proacrosine, esterases, phospholipase and non-specific acid proteinases in difference mammalian species. A combination of enzymes acts synergistically during the penetration process.

After sperm penetration through the zona pellucida the plasma membranes of sperm and ova undergo fusion. Acrosomal reaction is pre-requisite for this fusion. The sperm head travels up to the vitelline space and contacts the vitelline membrane. The perivitelline membrane is covered by dense microvilli except for an elevated area adjacent to the surface where the second polar body will be extruded after fertilization. Fusion of sperm and egg involve the plasma membrane over the equatorial segment and post-acrosomal region of the sperm head.

PREVENTION OF POLYSPERMY

Polyspermy is the fertilization of an ovum by more than one sperm. It prevents development of zygote. The sperm comes in contact with the ovum surface and several seconds later depolarization of the cell membrane of the ovum occurs and voltage inside the membrane increases. Within ~20 seconds cortical granules appear through cortical reaction, which is known as fertilization membrane. Release of cortical granules into the perivitelline space of the ovum, leads to reorganization of the entire zona pellucida. It provides a barrier for sperm penetration after entry of the first sperm and polyspermy is prevented.

MIXING OF THE GENETIC MATERIALS

Fertilization of the egg by the sperm completes the meiosis II. First and (or) second polar body is extruded into the perivitelline space. The remaining maternal haploid chromosomes are enclosed in female pronuclear. After fusion of the plasma membranes of sperm and ovum the spermatozoa nuclear envelop disintegrate and the released chromatin materials undergo decondensation. Male and female pronuclei migrate to the ovum center, the nuclear envelops disappear and the chromosomes are intermixed.

FERTILIZATION REGULATORS

Fertiliztion occurs under the influence of various stimulating and inhibiting factors. Izumo is found in mouse which is a protein with a single Ig domain. It may be found in the spermatozoa and facilitates fusion of sperm with ovum. In the ovum GPI linked compounds have been identified and tetraspanins like CD81 and CD9 have also been reported to facilitate fertilization in mouse.

Fertilization of mammalian ova is facilitated by calcium response which supports further development in a normal way. In some species the influx of calcium from outside is used to produce calcium signal, while in many species the calcium transportation is provided through release of calcium ions from stores inside the ovum. Several studies show that calcium stores may be located inside endoplasmic reticulum. Another cellular structure is involved in production of protein and lipids transportation of calcium ions across the cell membrane.

The immature oocyte of some animals like echiuran worms or bivalve molluscs the larger nucleus also called as germinal vesicle (GV) facilitates fertilization. The nucleus still remains arrested at meiosis prophase I. In many animals the germinal vesicle first undergoes breakdown and then completes maturation by resumption of meiosis.

Reorganization of the endoplasmic reticulum occurs with the maturation of ovum and it has been observed in living oocytes from mammals. The biological changes occurring during this process vary among species. Dil signal has been identified to be involved in fertilization in mouse oocyytes. These signals are diffused uniformly inside the ooplasm. After the ovum gets matured up to the status of metaphase II the Dil fluorescence move to cortical localization. In the oocytes of hamster oocytes the Dil first labeled many surface masses which increase in size after the breakdown of germinal breakdown. After complete maturation of the ovum, these masses disperse and constitute numerous clusters spread inside the ooplasm. It leads to production of a network of endoplasmic reticulum.

The fragmentation of reticulum after fertilization can not be explained exactly on the basis of existing information. However, studies on stem cells have revealed that increasing concentrations fo calcium support this process. Evidence from several studies suggest that in sea urchins an increase in calcium level in the zygote after fertilization lead to fragmentation of endoplasmic reticulum and wave like progression. This suggests that the raising calcium levels support the process of fragmentation. The fragmentation of

endoplasmic reticulum appears to support the process of migration of the pronucleus as well as the disintegration of the nucleus envelop of the sperm.

Studies have reveled that in mice endoplasmic reticulum lack reorganization after the process of fragmentation during fertilization. So the endoplasmic reticulum remains intact after fertilization. Detailed studies have shown that disassembling of the clusters fo endoplasmic reticulum occur during formation of the second polar body. It coincides with the 3rd to 4th hour of insemination and 2 hours before the inhibition of calcium signaling.

The current information suggest that during maturation and fertilization of the ovum, the endoplasmic reticulum experiences structural reorganizations. Further explorations are required for various animal species where fertilization completes before maturation of ovum. The mechanism of structural organization of endoplasmic reticulum need to be explored in detail. These structural organization in relation to the calcium signaling also need detailed studies on mature and immature ova.

CLEAVAGE

After fertilization the zygote floats freely in the fallopian tube. The zygote undergoes several mitotic divisions. Instead of dividing into separate cells, the divisions occur within the same zona pellucida, called as "cleavage". The daughter cells are called "blastomeres".

Due to the larger size of the zygote with low nuclear cytoplasmic ratio, size of the zygote remains almost constant while the cell division goes on. The first cleavage gives rise to two blastomeres, the second to four, the third to 8 and so on, in a doubling sequence. All these cell division are mitotic.

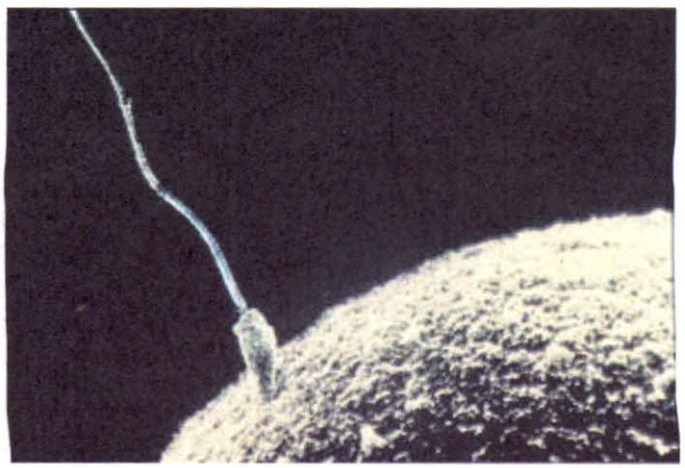


Figure 2.5 A sperm attempting to fertilize an egg **Source:** Wikipedia, Fertilization (*http://en.wikipedia.org/wiki/Fertilization*)

Up till 8 cell stage, the each blastomere possesses the capacity to develop into independent embryos. The blastomeres remain enclosed in the zona pellucida up to 16 cells stage and due to its mulberry like shape; it is called as "morula". After cleavage the zygote is called "embryo".

Unlike mitotic cell division in other cells, the cleavage occurs without going through the growth phase and the division continues in the daughter cells also without the growth phase.

The zygote gets its nutrients from the surrounding oviductal or uterine fluid. Size of the zygote may get increased slightly due to absorption of water. The blastocyt possesses a high degree of metabolic selectivity and control the entry of substances actively, from the surroundings; it is not a simple diffusion.

The embryo is activated genetically at the beginning of the cleavage. The activated genes are concerned with protein synthesis. The zona pellucida is lost either due to proteolytic enzymes of the estrogen sensitized uterus or progesterone, depending upon the species. The cell divisions are nearly but not exactly synchronous and uneven number of blastomeres may be seen. For example a three cell embryo occurs if the second cell division completes in one blastomere earlier than the other. Earlier dividing blastomeres are more likely to contribute more to the inner cells mass than the late dividing blastomeres which will contribute more to the trophoectoderm of the blastocyt.

FORMATION OF MORULA

Due to cleavage, the number of blastomeres increase but the overall size of the zygote remains almost constant. This is due to the fact that no growth phase of the cells is there and the cytoplasm of the one celled zygote is divided into many compartments until a solid mass of cells called "morula" is produced. In most of the species, the morula contains 16-32 blastomeres. The cytoplasm to nucleus ratio is highest in the one celled zygote and continues to decrease until it reaches to the ratio found in normal adult body cells. At this stage the morula converts to blastocyst.

Gap junctions are established for direct communication between the blastomeres incipient and tight junctions between the blastomeres on the outside of embryo. This is called "compaction". Compaction occurs at 8 cells stage in mouse, 16 celled stage in sheep and at 32 cells stage in cow. The outer cells become polarized and develop higher density of microvilli at the outer free surface. Inner cells remain apolar. Polar cells tend to contribute to formation of trophoectoderm and the apolar cell to the inner cells mass.

FORMATION OF BLASTULA

The morula hatches to form blastula or "blastocyst". The blastomeres start secreting fluids and position themselves around a central fluid filled cavity called blastocele. Blastocele formation is facilitated by sodium dependent active transportation of water into the blastocyst. The blastocyst cells are differentiated into two categories, the inner cell mass making the embryo proper and the trophoectoderm (or trophoblast) making the chorion of placenta.

TWINS FORMATION

After first cleavage of the zygote, if the two cells separate completely, monozygotic twins (identical) are derived. Dizygotic (fraternal/non-identical) siblings descend from different fertilized ova.

EMBRYOGENEIS

The gestation (pregnancy) stages have been defined as the zygote from 1-13 days, the embryo from 14 to 45 days and the fetus from 46 day up to parturition. However, under the popular embryo transfer technology, the word "embryo" is used for the zygote before hatching from the zona pellucida, which is transferable to the recipient for further development. Figure 2.6 shows embryogenesis

At various sites in the reproductive tract. Figure 2.7 shows embryonic development in various animals and 2.8 in bovines. In the preceding section, embryonic development up to blastula was described, which is hatched from the zona pellucida.

Blastocyst elongation follows the blastocyst development. The single layerd embryonic cells move to form two layers of cells through the process of gastrulation .

Germinal Layers Development begins at about 14th day, initiating the embryonic phase. The inner cell mass give rise to the three germ layers called as ectoderm, mesoderm and endoderm. Ectoderm is the outer layer which leads to formation of the external structures including skin, hairs mammary glands, hoves and nervous system. Mesoderm is the middle layer of cells giving rise to muscles, heart, and bones and the internal layer gives rise to the internal organs. At about day 16 the embryo sends signals of its presence for maintenance of pregnancy which results in continuation of luteal functioning up to parturition. On day 45 the formation of primary organs is completed beginning the fetal phase.

After hatching from the zona pellucida the denuded embryo (blastocyst) comes in direct contact with the uterine wall. It starts proliferating rapidly. The outer wall of the elongated blastocyst named as chorion gets apposed with the folded uterine wall. The embryo gets nutrients from histotrophe or uterine milk.

Shaping Up the Adult Organism

The zygote acquires the complex shape of the adult organism through a chain of events. Shaping up of the organism commences with the process of gastrulation. It brings changes in cell behavior converting the embryo into a trilaminar entity, comprising the outer layer of ectoderm inner to which lies the mesoderm. The innermost layer is the endoderm. The three cellular layers re-assemble the cells to form the organ and tissue parts which lead to formation of the functional organs and specialized tissues which are characteristic of the adult organism. Cells show changes in development, function and division process either independently or in coordination with the neighbors during formation of embryo. The cell function will be manifested in a different way when they function independently than when they act in coordination with their neighbouring cells. Morphogical and genetic changes occur involving mesenchymal and epithelial cells.

Embryogenesis in Various Species

The uterus of sheep and cow contains caruncles which are attachment organs for embryo. At about 16th day the expanded chorion fills up the entire uterus and trophoblast comes in contact with the caruncles. The trophoblastic cells eat away the uterine epithelium and establish contact with the maternal tissues. In cattle the chorion forms attachment with cotyledon at about 33 days. In mare during 10-14th week the chorionic villi penetrate the uterine wall and the attachment completes.

Regulation of Embryogenesis

Leukaemia inhibitory factor (LIF) has been found as a regulating element inhibiting conversion of embryonic stems cells into specialized cells in culture. It leads to retention of pluripotency of these cells enabling production of chimeric mice. The endometrial glands express mRNA in the mouse for LIF which occurs at the time of implantation of the blastocyst. The LIF receptors are found on blastocyts before implantation.

LIF expression originates from signals of maternal origin, which are probably regulated by changing levels of oestradiol. Murine and ovine blastocysts are supported by recombinant LIF in culture and the bioactive type of LIF has been reported to be species specific. Various stages have suggested that LIF acts upon trophoectoderm blastocyst which is rapidly expanding and implantation rate of the embryos is improved.

Quranic Wisdom

Creation of human beings from a mingled drop of fluid (*nutfa amshaj*) has been mentioned by the Holy Quran and explained by the Muhammad (SAW), the Prophet, precisely. The Holy Quran has mentioned; "*Verily We created man from a drop of a mingled fluid-drop nutfa amshaj), in order to try him: so We gave him (the gifts), of hearing and sight.*" (76:2)

Amshaj is a word used for mingling and it may be explained by mingling of spermatozoa form man with oviductal fluids of woman. This may be confirmed from speeches of the Prophet Muhammad (SAW). It has been mentioned by ***Imam Ahmed in the book "Al Musnad" that Mohammed*** (SAW), the prophet ***was speaking to his companions, when a Jew passed nearby. A few persons of the Quresh tribe told a Jew that that man has claimed to be a prophet. The Jew told them that he will ask him some questions which no one can answer without a prophet. The asked the prophet*** (PBUH) ***that what a man was created from. Muhammad*** *(PBUH), the prophet* ***said that man is created from two fluids, the man's nutfa and the woman's fluid. The Jew informed that he is right and the same has been said by the prophets, who came earlier.***

The embryonic development has been described by the Quran as: "then of that fluid-drop (nutfa) we created a leech-like clot" (23:14). Alaqa is the stage of embryonic development that starts on 15th day and remains up to 23rd or 24th day. Afterwards the embryo developed gradually and takes it shape like a leech living in the ponds. Alaqa gets attached to uterus through umbilicus.

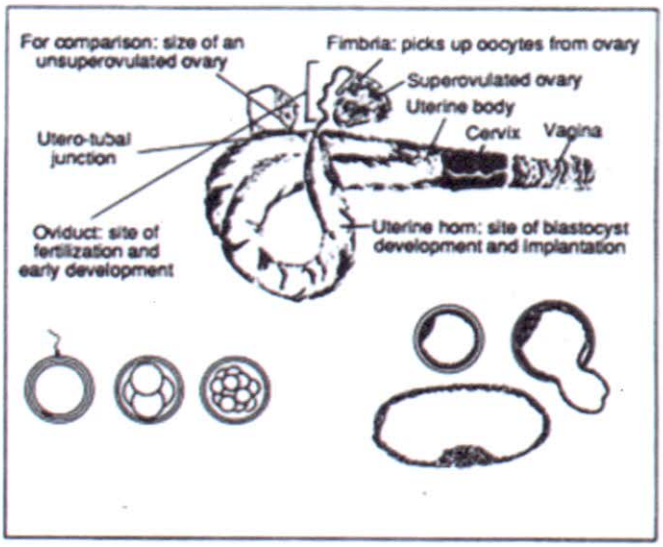

For comparison: size of an unsuperovulated ovary
Fimbria: picks up oocytes from ovary
Superovulated ovary
Uterine body
Cervix
Vagina
Utero-tubal junction
Oviduct: site of fertilization and early development
Uterine horn: site of blastocyst development and implantation

Figure 2.6 Embryogenesis at various sites in the reproductive tract
Source: Embryo transfer in cattle. FAO, 1991

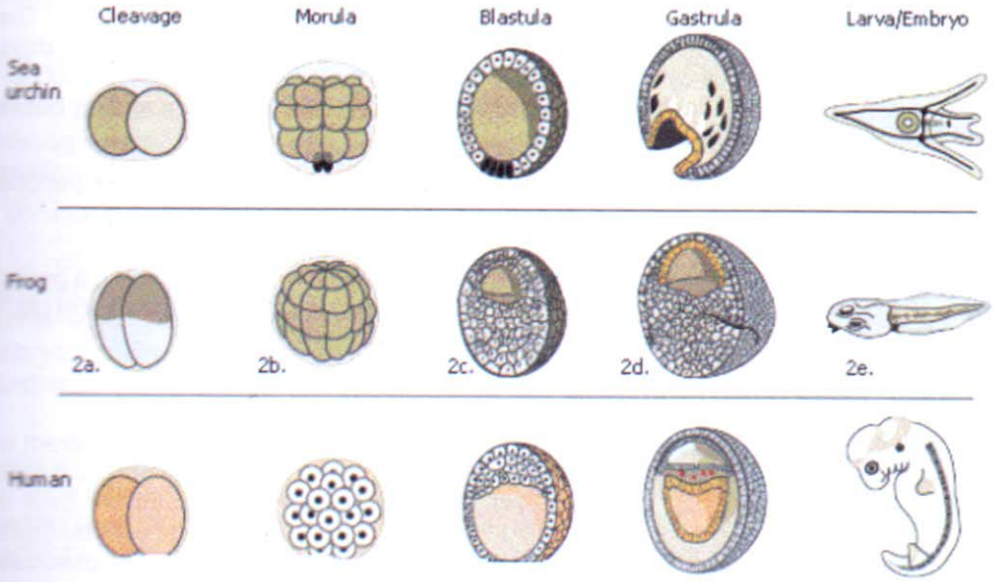


Figure 2.7 Embryonic development in various animals.
Source: Microsoft Encarta Encyclopedia 2005

MATERNAL RECOGNITION OF PREGNANCY

Estrous cycle continues after ovulation, up to the next estrus and ovulation. The corpus luteum developed after ovulation, continues functioning throughout the luteal phase of the estrous cycle. In case of non-pregnancy, the uterus secretes prostaglandin, which causes regression of the corpus luteum, decreasing levels of progesterone, commencement of folliculogenesis, raising estrogen levels and occurrence of estrus once again.

In case of pregnancy the corpus luteum continues functioning, maintenance of higher blood progesterone levels and cessation of estrus cycle at the luteal phase, until parturition. The phenomenon of signaling the dam by the embryo for its presence and continuation of the luteal phase up to parturition is known as maternal recognition of pregnancy. This signal is necessary for development and secretary activity of the endometrium. If this signal fails to occur, the pregnancy will be terminated through luteolysis caused by prostaglandin from the uterus. The signal blocks production of prostaglandin from the endometrium, thus preventing luteolysis and allowing pregnancy to continue.

The effect of conceptus is luteostatic since production of progesterone is maintained at levels comparable with the luteal phase of the estrus cycle. For maintenance of pregnancy and higher levels of progesterone, the basal levels of the LH are also necessary. An early pregnancy factor (EPF or early conception factor, ECF) has been identified in various mammals a few hours after mating, preventing recognition of embryonic antigen.

SPECIES DIFFERENCE

In cows the maternal recognition of pregnancy occurs during 16 to 19 days of pregnancy. The bovine conceptus produces low molecular weight acidic proteins. These proteins when introduced into the uterus of non-pregnant cows between 15 to 21 days of estrus extended the estrous cycle by 8 days. In ewes the maternal recognition of pregnancy occurs on day 12. In non-pregnant gilts the prostaglandin secretions increases between days 14 to 15.

Pregnant mares have little $PGF_{2\alpha}$ in uterine fluids. $PGF_{2\alpha}$ in peripheral plasma shows no episodic pattern of release. However, in the presence of conceptus the $PGF_{2\alpha}$ secretion is significantly low. In gilts the maternal recognition of pregnancy occurs on day 11 to 12. Later on estrogen is produced during a period of 16 to 39 days after pregnancy. During pregnancy and pseudo-pregnancy in gilt, the $PGF_{2\alpha}$ is significantly lower in blood circulation than cycling gilts.

MATERNAL EMBRYO INTERACTION

During implantation of embryo with the uterine wall the maternal endometrium interact with the embryo, manifested by mediation through various molecules and signaling cascades. However, these phenomenon have not yet been fully understood. Stromal cells are transformed into decidual cells know as decidualization, during this chain of events. Studies on baboon have shown that presence of the conceptus regulate the changes associated

With these events. The implanting embryos have been shown to produce cytokines during the process of implantation.

Interleukin-1 system has been found to contribute into the process of decidualization. The endometrial cells, after decidualization have been found to contribute to expression of gene products helping in establishment of pregnancy. The decidual cells produce an insulin-like growth factor binding protein-1. It facilitates action of decidua with the invading trophoblast. The enlightenment of the interaction among transcription factors FKHR and HOXA10 followed by expression of IGFBP-1 and the relationship among IL-1 and IGF cascades need to be explored to explain the biology of successful implantation of embryo with the uterine wall.

Presence of corpus luteum on the ovaries of cows is not essential as in cows having no corpus luteum have been reported to maintain pregnancy with exogenous progestogen administration. A unilateral relationship has been found between the fetus and the corpus luteum leading to maternal recognition of pregnancy. A study has reported successful maintenance of pregnancy in luteotomized cows in which a corpus luteum was induced during second month post-conception, on the side of occurrence of pregnancy. Maintenance of pregnancy increased from 50% to 100% when the CL induction was delayed from day 28 to 35.

Studies have shown that a corpus luteum developed on cow's ovary on 53rd day of gestation through hCG administration, maintained pregnancy up to parturition. The corpus luteum of this cow was removed and replaced by exogenous progesterone administration. Evidences from the previous studies suggest that the association between the developing conceptus and the corpus luteum seem to disappear by the third month of gestation.

SEX DIFFERENTIATION

The Bi-Potential Gonad is the common precursor of mammalian gonads during embryogenesis. It possesses the capacity of developing into either testis or ovary. Genetically the sex is determined during fertilization as an ovum can either be fertilized with Y-bearing spermatozoa, leading to a male embryo or an X-bearing spermatozoon leading to formation of a female embryo. However, the bipotential gonad still retains the capacity to lead to formation of testis or ovaries and it is driven by the presence of Y chromosome and the expression of Y-linked gene *Sry*. The *Sry* gene in the male embryo stimulates a cascade of events which leads to development of testis and suppression of ovaries. In the female embryo the *Sry* gene is absent and the bi-potential gonad converts into ovary. Fig. 2.9 illustrates the determination of sex through changes in the structure of gonads.

After conception the male and female embryonic gentical ducts (Wolffian and Müllerian ducts) are found in the bi-potential gonad up to 11th day. With the expression of *Sry* gene the bipotential gonad develops into male gonad and testicular hormones are produced, leading to regression of Müllerian ducts and subsequently the Wolffian duct develops into epididymis, *vas deferens* and seminiferous tubules, and testicular descent.

In the females embryos the *Sry* is absent and the Wolffian duct regresses, leading to formation of ovary and conversion of the Müllerian ducts into oviducts, uterus, and anterior

Portion of vagina. In males, the presence and expression of *Sry* leads to formation of the spermatic cord, coelomic vessel and multiplication of cells

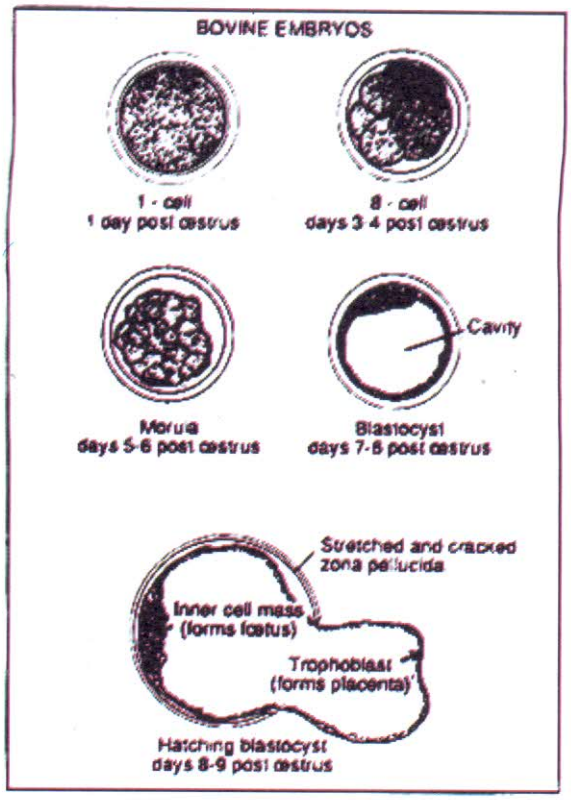


Fig. 2.8 Development of bovine embryo (Source: FAO, 1991)

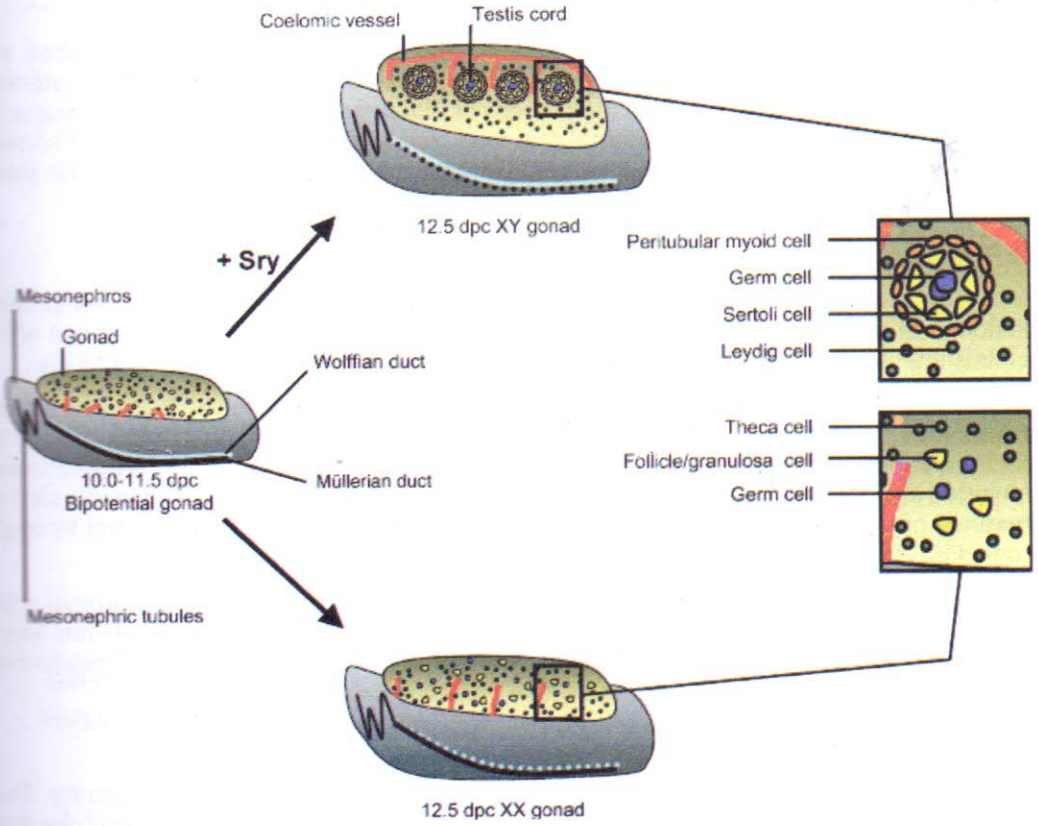


Fig. 2.9 Structural changes in the gonad following sex determination (Bernard and Harley, 2006)

Several genes have been found up and downstream in studies on mouse related to engineered mutations in mice, regarding expression of *Sry* gene regulating sex determination leading to development of genital organs.

Quranic Description of the determination of sex during development of the embryos comprises transformation of alaqa to mudgha, in a rapid way completing within two days, 24th to 26th day post-conception. The rapid change has been expressed with the fa, meaning 'then' and pointing at the rapid development of transformation. The holy Quran says, "then We changed the Alaqa (leech-like clot) into a Mudgha (chewed-like lump) " (23: 14). We can observe that different conjunctive articles used by the holy Quran which reflects the difference in embryonic stages. The alaqa has been mentioned as second stage in embryonic development and described in several verses. He the Almighty says; "Was he not a drop of sperm emitted (in lowly form)? Then did he become a leech-like clot; then did (Allah) make and fashion (him) in due proportion. And of him He made two sexes, male and female."(75: 37-39).

TPLACENTATION

After fertilization, cleavage and blastulation, mammalian embryo establishes an intimate relationship with its mother. The trophoblast lineage differentiate resulting in uterine implantation and commencement of the process of **placentation.** Inter-hemal membrane is a vital layer that separates the maternal and fetal blood from each other. Fig. 2.10 shows histology of placenta. Fig. 2.11 shows placenta attachment in ruminants and Fig. 2.11 in sow. Table 2.4 shows functions of placenta.

MECHANISM OF IMPLANTATION

It is surprisingly to note that trophoectoderm differentiation of equipotent blastomeres at stage of morula has not been made clear till now; but maintenance after establishment of the lineage of trophoblast stem cells, has been investigated extensively.

Multiple molecules and a series of biological reactions are involved in the interaction of endometrium with the embryo during the process of implantation. These biological reactions constitute the signaling cascades. During this series of events the stromal cells are converted into decidual cells known as decidualization. In the baboon stromal fibroblasts the presence of embryo is essential. It has been found that this process is mediated through production of cytokines by the implanting embryo.

Various gene products are expressed by the decidualized endometrial stromal cells which may lead to establish pregnancy. Insulin-like growth factor binding protein-1 has been found as the major products of decidual cells. This factor facilitates the function of insulin-like growth factor (IGF) protein.

SPECIES VARIATION

"Implantation" occurs in higher primates and rodents during early pregnancy. The blastocyt becomes rapidly embedded in the uterine wall and is on contact with vascular and connective tissues from all sides. "Attachment" occurs in domestic species where the conceptus remains within the uterine lumen and the surrounding allanto-chorion requires a close relationship with the endometrium over some or its entire surface. Placenta is also classified according to shape of the main area of the hemotrophic exchange. In discoid placenta it is disc like (human, monkeys); where it forms an equatorial zone around the placenta, it is zonary (dog, cat) and where the entire chorion is equally involved, the placenta is diffused (mare, pig). Where the principal area of exchange is fairly small, discrete and scattered, the placenta is called cotyledonary (cow, buffaloes, goat, sheep). At parturition the maternal tissue is lost in deciduate but not in non-deciduate placentation.

Table 2.4 Functions of placenta

Yolk sac: Provides nutrient supply to embryo during earlier stages.

Amnion: Provides protection to the fetus from injury and lubrication during parturition, functions as a reservoir for urine and other wastes.

Allantois: It fuses with chorion leading to formation of chorio-allantoic placenta, carries blood vessels of placenta, reservoir for nutrients and wastes. Umbilical cord attaches fetus to allantois.

Chorion: Attaches to uterus, absorbs nutrients from uterus, allows maternal/fetal gas exchange, and produces hormones.

Placenta * Substitute for the fetal digestive tract, lungs, kidneys, liver and endocrine glands and separates maternal and fetal organisms.

* Permits transportation of sugars, amino acids, vitamins & minerals as substrates to the fetus, serves as a storage organ for glycogen, iron

* Produces eCG (PMSG), progesterone and estrogens

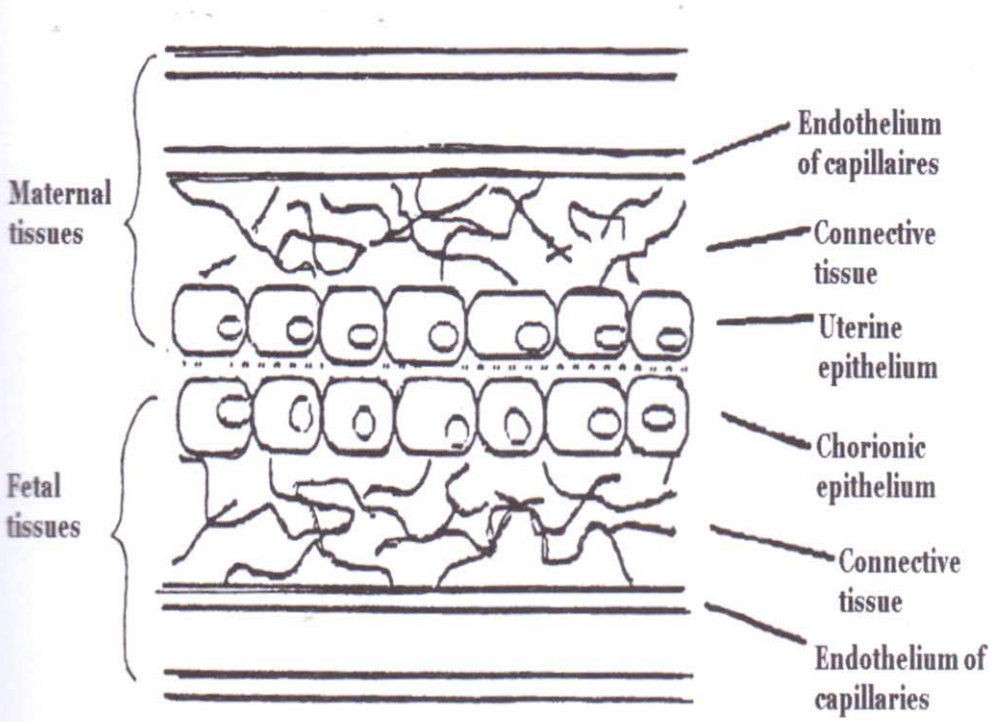


Fig. 2.10 Histology of placenta
Source: Cornell University, 2006

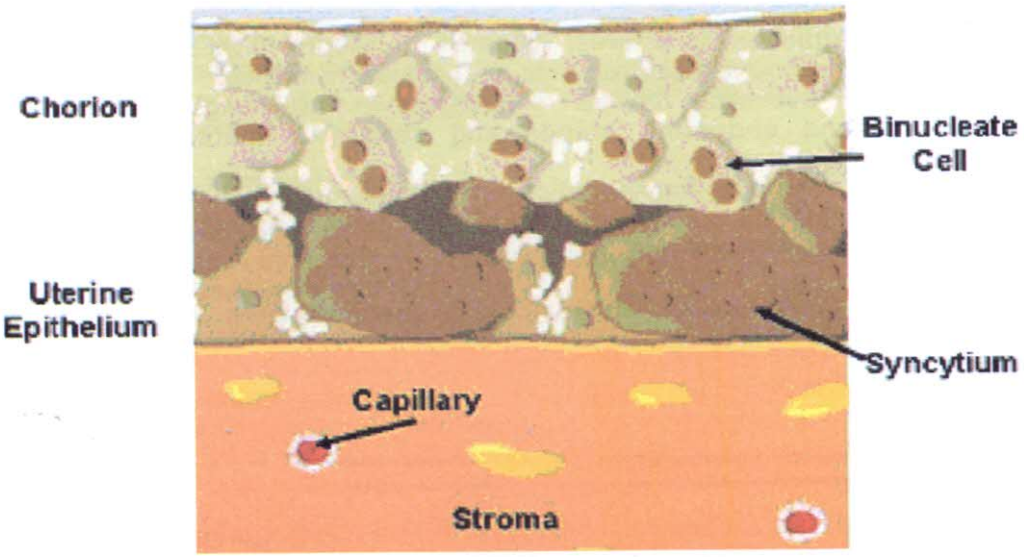


Fig. 2.11 Placenta attachment in ruminants
Source: Geisert, 1999.

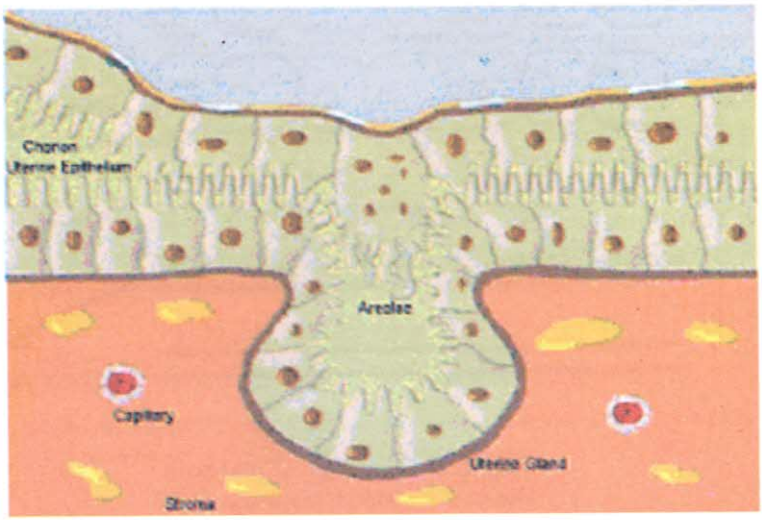


Fig. 2.12 Placental attachment in sow
Source: Geisert, 1999.

Fig. 2.13 Diffused, zonary, cotyledonary and discoid placentation
Source: Hafez, 1987

GESTATION AND PARTURITION

GESTATION

Mammals are viviparous and give birth to young one at the end of gestation *period comprising the embryonic and fetal development inside the uterus. Gestation or pregnancy provides nutrition to the growing fetus and adaptation of mother.*

Length of Gestation is the interval from the fertile service *up to parturition. It* varies with the species and is almost constant within the species. Slight variations exist among the breeds. Gestation period of cattle is about 280 days ranging from 240 to 310 days in dairy cattle and from 243 to 316 in beef cattle. Gestation length of buffaloes is longer than cattle (315 days, ranging form 305 to 330 days). In sheep, the gestation period is 148 days ranging from 140 to 159 days. In swine the gestation period is 114 days, ranging from 102 to 128 in domestic swine and 124 to 140 in wild swine. In horses the gestation period varies from 334 to 344 days.

Age of the dam is inversely proportional to gestation length and younger cows show a gestation length of 2 days smaller while in older cows the length is slightly longer. Larger litter size shortens gestation length in polytocus species. Male calves and foals are carried 2-3 days longer than females. Smaller variation in gestation length may be due to genetic, seasonal or local factors. Extremely prolonged gestation may be seen in dairy cattle carrying fetus homozygous for autosomal recessive gene.

CHANGES IN MATERNAL ORGANS

During the late gestation changes occur in the reproductive tract of the mammals. The vulva and vagina become edematous and vascular, especially in cattle and buffaloes. The external os of the cervix is tightly closed and a secretion of highly viscid mucus seals the cervical canal, known as the "mucus plug of pregnancy".

The uterus undergoes gradual changes to accommodate the growing fetus but the myometrium remains quiescent thereby preventing premature expulsion. Endometrial proliferation occurs before blastocyst attachment and is caused by preparatory progesterone sensitization of the endometrium.

The ovary carries a corpus luteum known as the corpus luteum verum which remains throughout pregnancy. It secretes progesterone which is the main hormone for maintenance of pregnancy. It prevents folliculogenesis through a negative feedback on hypothalamus and pituitary. Therefore, occurrence of estrus is suspended. However, in mares follicles develop during 40^{th} to 160^{th} day of pregnancy which ovulate and lead to formation of more corpora lutea assisting in maintenance of pregnancy. The source of progesterone during the latter half of gestation is placenta in mare and ewe and CL in cow, goat and sow.

Relaxation of pelvic ligaments occur during advancement of pregnancy and accelerate during the period of approaching parturition.

This has been attributed to the higher levels of estrogens and the relaxin during late pregnancy.

MOTHER-FETUS INTERACTION

The fetus carries genetic materials from the mother as well as father. The genetic material inherited form father is considered as foreign material for the uterine environment of the mother. So the fetal tissues are considered as an allograft or tissue from other individuals of the same species. If the donor possesses an antigen that is not possessed by the recipient, recipient will reject the transplanted tissue.

Antigens that provoke rejection are called transplantation or histocompatibility antigens. The rejection is usually mediated by T-lymphocytes rather than antibodies. These transplantation antigens are present on the cell surface and are directly exposed to the T-lymphocytes of the circulatory system of the recipient.

The maternal immune system is antigenically challenged by the fetus that should be capable of eliciting immunologic rejection reactions, but unlike immunologic destruction of allograft in other parts of the organism, the placenta is not rejected until parturition.

Failure of maternal tissue to reject the placenta has puzzled immunologists and has led to a number of theories to explain the unique relationship between mother and fetus. One hypothesis assumes ***fetal antigenic immaturity*** which can be rejected due to demonstration of transplantation antigen in the embryo. ***Maternal immuno-suppression*** has been presumed, however, removal of fetus from the uterus and its transplantation in flanks region of the mother leads to allograft reaction. Some workers have described uterus as an ***immunologically privileged site*** which is not true as oviduct, anterior chamber of eye or peritoneum can support normal fetal development. ***Immunologic barrier*** is another presumption for survival of fetus in the uterus. The sialomucin (fibrin) layer that exists between the trophoblast cells and maternal tissues in most chorioallentoic placentas. But the fetal and maternal cells cross this fibrinoid barrier and survive in the hostile environment. Also the layer is not continuous between maternal and fetal tissues it is absent around the villi of human placenta.

The growth and development of fetus is supported by establishment of multiple pathways during pregnancy. These pathways help in communication of mother with fetus. A series of enzymic reactions is followed by emergance of phospholipid-derived mediators. The formation of PDM be followed by synthesis of secondary mediators. Prostaglandins have been found as important regulators in permitting pregnancy and parturition in the mammals.

PARTURITION

Parturition is the physiological process of expulsion of fetus and the placenta by the uterus from the maternal organism. Two prerequisites for the process of parturition are the ability of the uterus to push out the fetus and placenta and the ability of cervix to dilate sufficiently for passage of the fetus. Fig. 2.14 shows factors affecting fetal growth in farm animals. Fig. 2.15 shows endocrine profile of parturition in farm animals and Fig. 2.16 shows sequence of events in parturition in sheep.

SYMPTOMS OF PARTURITION

One or two weeks before parturition, certain signs are exhibited by the animal which helps in predicted the date and time of parturition. These symptoms are developed under the influence of hormonal changes originated form the fetal or maternal source.

The vulva becomes edematous, pelvic ligaments show changes and the udders become enlarged. In all farm species, the teats become swollen and secretion may escapes from the teat orifice. In mare the colostrums oozes from teat orifice and form a bead of waxing material at each teat orifice, between 6 to 48 hours before foaling, replaced by drips of milk 12 hours later. The animal seeks isolation before parturition.

THE PROCESS OF PARTURITION

The process of parturition comprise of uterine contraction and dilatation of cervix resulting in expulsion of fetus from the uterus. Uterine contraction occurs under the influence of estrogen and prostaglandins and cervical dilatation occurs under the influence of estrogen, prostaglandin and relaxin.

Uterus remains under the influence of progesterone during the gestation period, secreted by the corpus luteum. It provides an environment conducive for development of the fetus. Low amplitude myometrial contractions take place throughout the gestation period. However, with the onset of parturition the intensity of the contractions increase, characteristic of labor. The declining levels of progesterone and increasing levels of estrogen are associated with the myometrial contraction. Higher levels of progesterone during pregnancy block the myometrial contractions and the declining levels remove the block, resulting in myometrial contraction. Prostaglandin is released from the uterus which interacts with the smooth muscle adenyle cyclase system to lower cAMP levels leading to myometrial contraction.

Cervix remains closed during pregnancy sealing the conceptus through the higher contents of connective tissues (collagen). A few hours before the labor contraction, the cervix softens, becomes more compliant and gradually dilates.

STAGES OF PARTURITION

Dilation of cervix remains for up to 2-6 hours in cows. Uterine contractions become coordinated and regular which are estrogen & PGF2α induced. Fetus is pushed against cervix and amnion dilates the cervix. Allanto-chorion may break at this stage. Pressure of fetus in cervix stimulates oxytocin release and reflex contractions of abdominal muscles.

Expulsion of fetus follows remaining up to 2-5 hours in cow. Strong uterine contractions follow due to synergistic actions of high estrogen, PGF2α and oxytocin. Strong abdominal muscle contractions follow. Amnion ruptures - mucin lubricates vagina and vestibule. Fetus passes through vagina and the vestibule.

Expulsion of the placenta is the next stage remaining for 4-5 hours in cow. Uterine contractions continue. Blood is forced from cotyledon villi - shrinkage separates cotyledon and caruncle. Contractions push placenta out. Placentas may be retained due

to infections causing adhesions between cotyledons and caruncles; calving stress (twins or calving difficulty, edema of cotyledon preventing cotyledons' separation from caruncle) and weak uterine contractions where villi won't shrink.

Uterine involution is the last stage. Cow passes lochia for 1 to 2 weeks after birth comprising mucus, blood, fetal membranes and surface of caruncle. Caruncles are regenerated by ~ 30 days post calving. Uterus is completely involuted by 45 days postpartum. Shrinking of uterus occurs due to muscle contractions. Involution is enhanced by suckling where oxytocin is released and by estrus periods when estrogen levels are high in blood. Foal heat occurs 10-15 days postpartum in mare with low fertility due to lack of involution.

HORMONAL PROFILES

Progesterone remains higher during pregnancy and declines with the onset of parturition. Source of progesterone during pregnancy is CL or placenta. In mare the progesterone levels remain higher at this time also. In CL dependant species (cattle, goats and pig) the parturition is initiated through regression of CL. In placenta dependent species (sheep), fetal cortisol activates a steroid 17-α hydroxylase an enzyme that converts progesterone into estrogen.

Stimulation of $PGF_{2\alpha}$ occurs at the onset of parturition through declining level of progesterone and rising levels of estrogens. Evidence suggests the $PGF_{2\alpha}$ plays a central role in inducing strong uterine contraction of the second stage of labor and the cervical dilation. Oxytocin levels show little change during initiation of labor but the levels increase significantly during the expulsion of fetus in most farm species. Oxytocin mediates the neuro-humoral reflex ***Ferguson's reflex*** during labor, in which the conceptus stimulates the birth canal.

INITIATION OF PARTURITION

Extensive studies have been conducted on various mammals; however, the complete biological pathway for pregnancy maintenance and commencement of parturition could not be known in any species till now. Various studies have reported variation in the pathways in various species and orders of animals. The physiological processes involved in reproductive events indicate that these event are not regulated by a unified factor. It has been suggested that pregnancy maintenance and commencement of parturition are regulated by a diversified type of factors instead of a unified regulator.

Attempts to relate physiological and experimental observations on the initiation of normal and induced parturition in experimental animals to events occurring in the human have made extensive use of the sheep as an experimental model. Such observations have been supplemented by a small number of studies in non-human primates and, where possible, the human. Animal models, however, have been used to great effect in the study of the development and the treatment of abnormal development in the human.

The maternal blood circulation provides substrates for the developing fetus. With the increasing fetal age these requirements also increase. In sheep and some other species it has been shown that requirements of the fetal development exceeds the availability of substrates during advanced gestations stage and the placenta is not able to meet these

Requirements. This phenomenon induces a stress factor which activates the fetal hypothalamo-pituitary-adrenal (HPA) axis, leading to commencement of parturition in some species. The substrates deprivation is gradual negating commencement of parturition as a precise time, however, deprivation of substrates does result in commencement of parturition.

The time of parturition seems to be gene regulated, integrated into genome and the developmental events activate the gene followed by parturition.

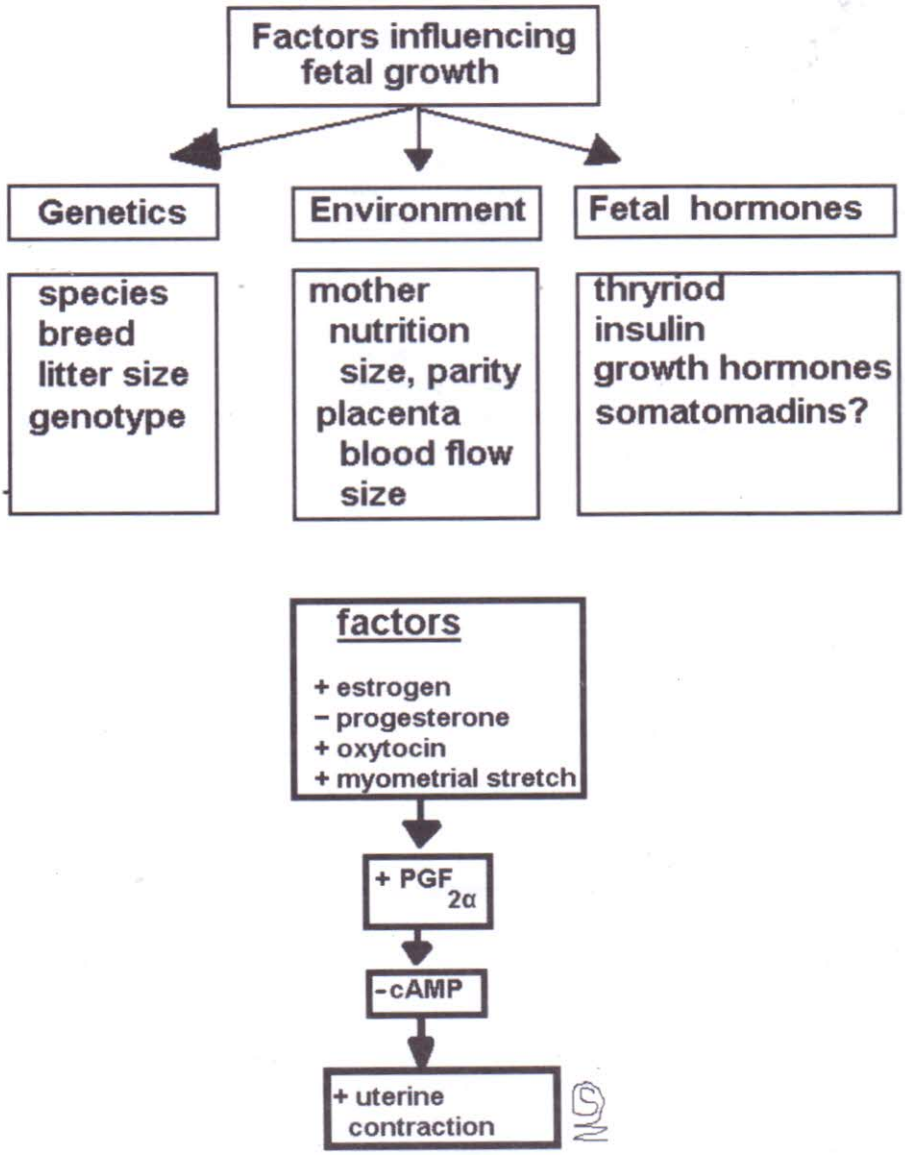


Fig. 2.15 Hormonal changes during of parturition in farm animals. **Source: Hafez, 1987**

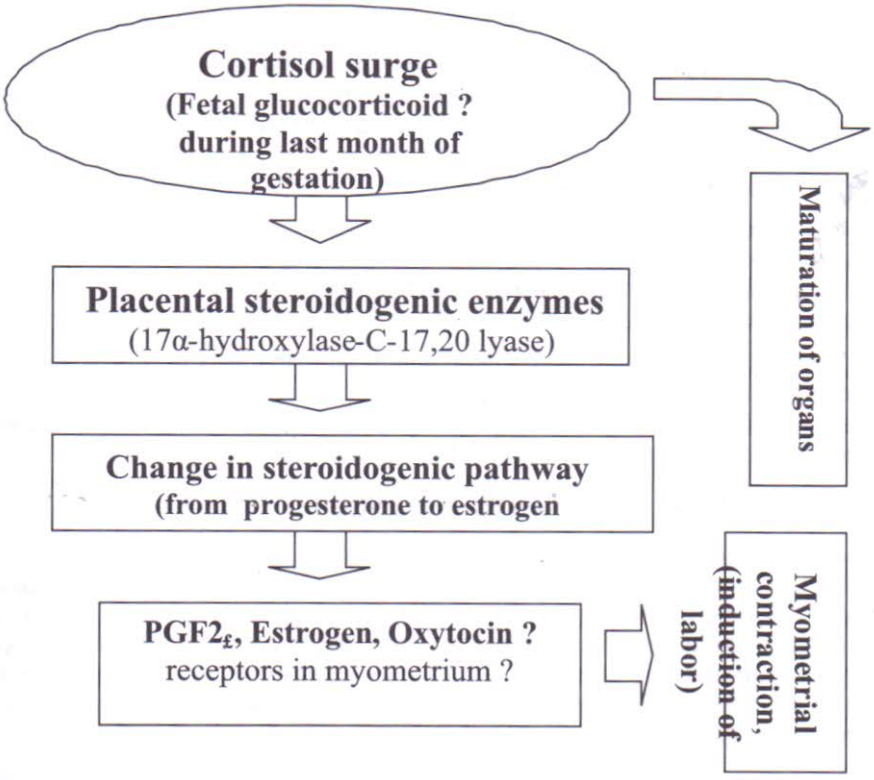


Fig. 2.16 Sequence of events in parturition in sheep.

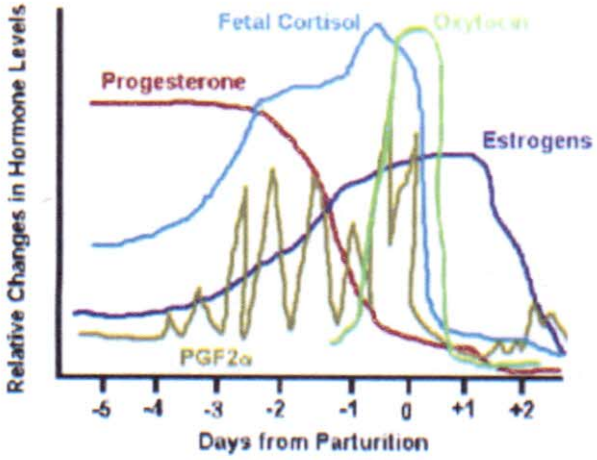


Fig. 2.17 Endocrinological profiles around parturition
Source: Geisert (1999)

LACTATION

The basic microscopic and gross anatomical structures of the mammary glands of mammals are remarkably similar. The only major difference among species is in gross anatomy associated with the removal of milk gathered in the major ducts.

DEVELOPMENT OF MAMMARY GLANDS

In bovine fetus, epidermal thickenings called mammary buds are formed on the milk line in the inguinal region between the hind limbs. The mammary buds sink into the mesenchyme and a condensation of mesenchymal cells takes place around the bud causing appearance of the embryonic teat hillocks.

During later stages the mesenchyme differentiate to form the connective tissue that will support the fully developed mammary gland, the connective glandular stroma that will ultimately surround the lobuloalveolar structure of the mammary gland, and adipose tissues that surround glandular elements and bulk of the udder.

PRE-PARTUM AND EARLY POSTPARTUM CHANGES

During the prepartum period mammary glands attain maximum development. A relative reduction in stromal cells and a concomitant increase in ductal and lobuloalveolar tissues occur. Mammary glands growth, as indicated by changes in total DNA accelerates exponentially throughout pregnancy n guinea pigs, goats and cows. This development is dependent upon estrogen, progesterone and pituitary hormones. Administration of estrogen progesterone combinations for prolonged periods, have been successfully used to induce udder growth followed by triggering doses of estrogen and initiation of milking.

During prepartum udder development the estrogen stimulates duct growth and progesterone regulates lobuloalveolar development. It has been shown that little or no mammary development occur prepartum unless anterior pituitary extracts are supplied. The anterior pituitary perhaps supports mammary development through production of estrogen and progesterone. However, direct action of the pituitary hormones on mammary gland has been demonstrated.

After a marked increase in the lobuloalveolar structures and cell numbers during pregnancy, three events occur which are necessary for lactogenesis: i) functionally differentiated secretory cells are formed; ii) development of these cells and; iii) expression of the capacity for milk synthesis.

Prolactin appears to fulfill functions essential for secretory cells proliferation and differentiation during late pregnancy. In post-mitotic or differentiated mammary cells the prolactin fulfills a central role in regulation of RNA and protein synthesis and thus is essential for the development of capacity for milk component synthesis within the newly formed cells. Glucocorticoids regulate synthesis of various enzymes essential for milk biosynthesis and thus along with insulin, are essential for development of the external rough endoplasmic reticulum of the fully developed mammary cells.

LACTATION AND IMMUNOSUPPRESSION

During the early phase of lactation majority of the cases of clinical mastitis develop. Several studies have reported the changes in various blood metabolites and hormonal concentrations around parturition. Enhanced susceptibility to mastitis have been found to be associated with systemic fluctuations in retinoid and steroid hormones. However, the mechanisms of immuno-suppression around parturition could not be explained clearly.

After invasion of bacteria the mammary epithelial cells and the macrophages help in causing inflammatory events targeted at elimination of the bacteria. It has been suggested that the mammary epithelial cells may produce cytokines. Interleukin-8 may be produced by mammary cells which may lead to increase in the number of polymorphonuclear neutrophil leukocytes in the milk. These cells constitute the major post-infection innate immune state.

Growth and apoptosis of various types of cells like mammary epithelial cells and polymorphonuclear neutrophil leukocytes are affected by retinoids. Retinoids have also been found to have a role in regulation of the immune system. Defects in the human immune system have been reported due to vitamin A deficiency. Research studies in mice support these observations.

LACTATION MANAGEMENT

The dairy farmers have been attempting to enhance farm profits through enhancing the level of milk production and decreasing feeding cost, ignoring the cost on management of health and fertility. The production of milk per head has increased over the previous decades which have been made possible due to intensive genetic selection supported by improved nutrition and management.

The increased milk productivity of dairy animals has been associated with concurrent enhancement of the level of health and fertility disorders. This deteriorated status of health and fertility has decreased the profit margins due to increase in the treatment cost, higher rate of replacement of dairy animals, decreased milk yield and quality.

LACTATION AFFECT ON HEALTH AND REPRODUCTION

There is mounting concern about a concomitant increase in health and fertility problems with the increased milk yield. The increase in health and reproductive problems may, at least to some extent, decrease the benefits of increased yield through reductions in the amount and quality of saleable milk and increased costs associated with veterinary treatments and replacement animals.

Increased productivity has resulted in an increase in the incidence of production diseases which have been studies during the previous decades. However, in spite of the preventive measures the risk of production diseases still higher probably due to the higher productivity boosted by genetic improvement and management strategies.

During earlier lactation the demand for nutrients increases substantially. For production of a milk yield of 50 kg the amount of milk fat secreted is about 2 kg daily,

protein 1.6 kg, lactose 2.5 kg, calcium 65 g, phosphorus 50 g and magnesium 8 g. It necessitates the intake of energy, protein and minerals. The requirements for nutrients during advanced pregnancy and lactation must be coordinated with the biological processes involved in fetal growth, milk production, maintenance of health and fertility.

Improvement in management practices have been introduced at herd level like feeding strategy. It has led to improvement in the health status of the herds. The strategy of maintaining more homogeneous groups of animals has helped in identifying risk animals, for reproductive problems, lower milk production, metabolic or immune disorders (Fig. 2.17).

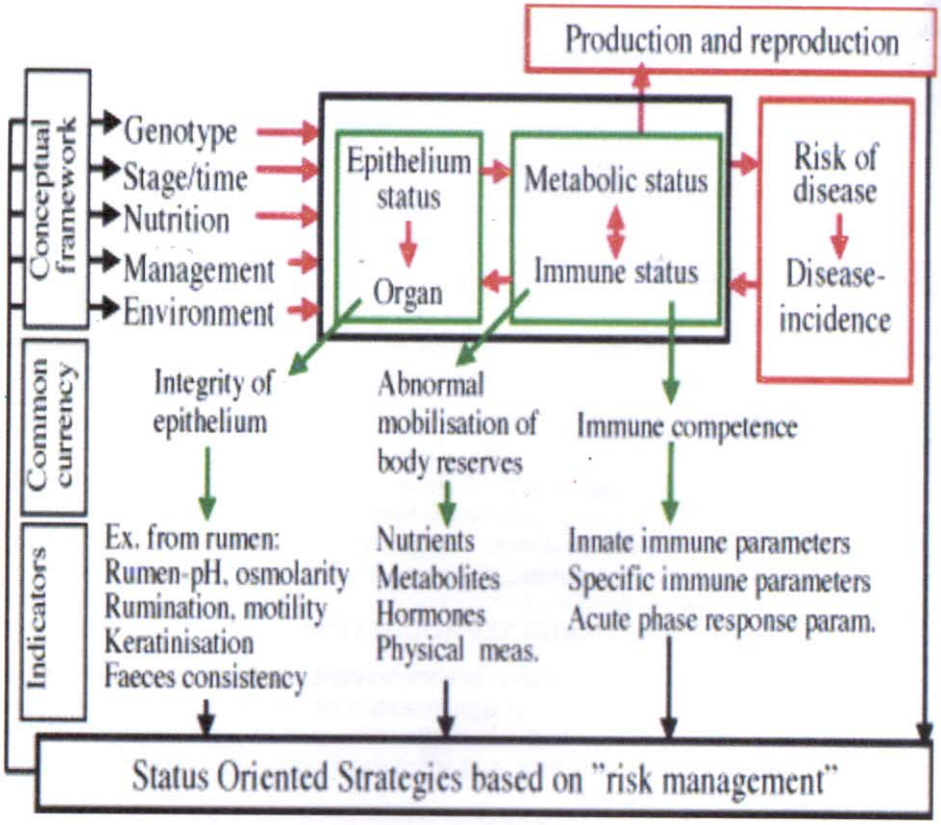


Fig. 2.18 Improving health, reproduction and productivity of dairy animals through improved management strategies Source: Ingvartsen, 2006.

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CHAPTER-3

ANATOMY AND PHYSIOLOGY OF MALE REPRODUCTIVE SYSTEM

INTRODUCTION

The male reproductive organs in mammalian species contribute in production and maintenance of supply of spermatozoa, detection of females in estrus and insemination of females. The spermatozoa reach the fertilization site and fertilize the female gamete. The male reproductive system comprises the testes, accessory glands, various ducts and the penis (Fig. 3.1)

THE MALE REPRODUCTIVE TRACT

Testis is the primary gonad of mammalian males and produces spermatozoa as well as testosterone (Fig. 3.2). Testes are two in number and lie outside the abdominal wall, in the scrotum, which is an extension of the skin. The extension of peritoneum holding the testis is called the vaginal process. Blood vessels and nerves reach the testes through spermatic cord lying in the vaginal process. The vaginal process passes through the inguinal canal. The testis is secured to the wall of the vaginal process along the line of its epididymal attachment.

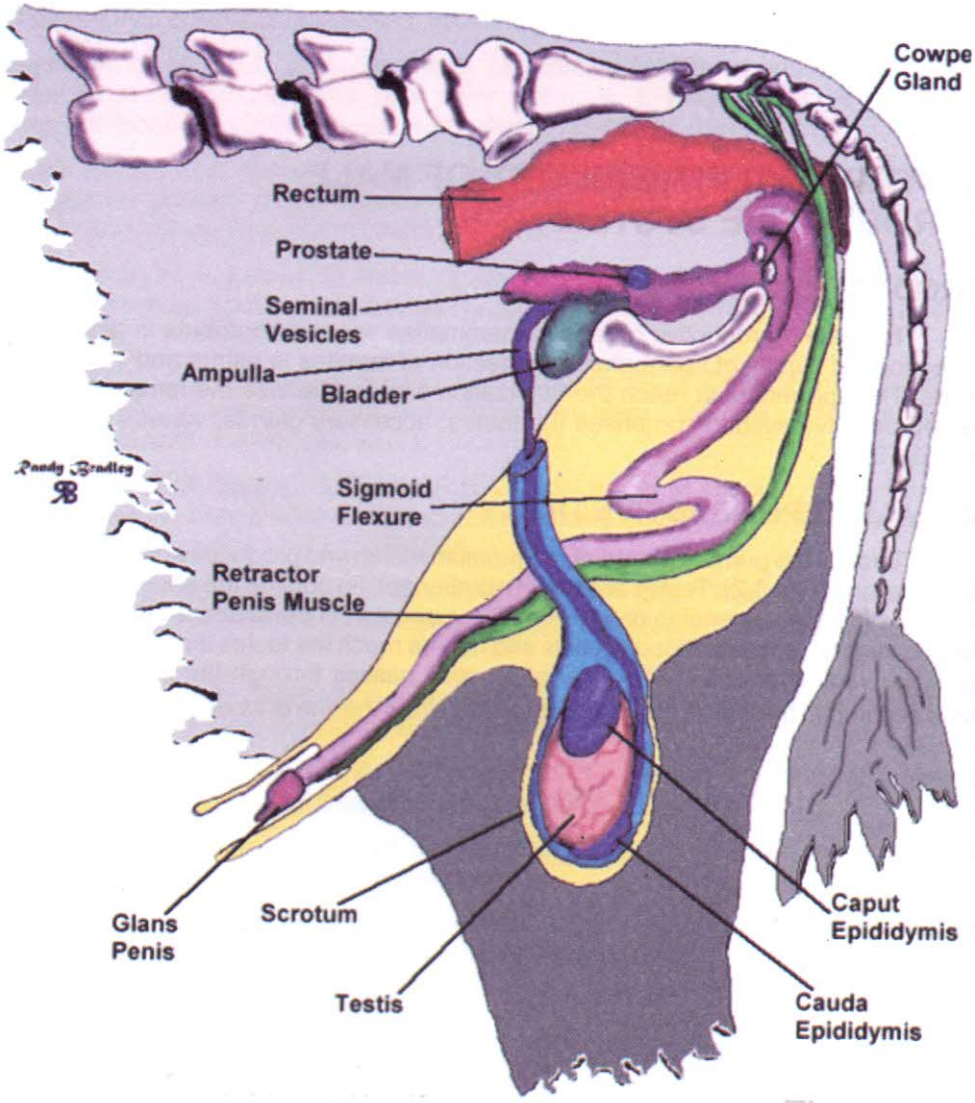


Fig. 3.1 The male reproductive tract of bull.

Source: Geisert, 1999

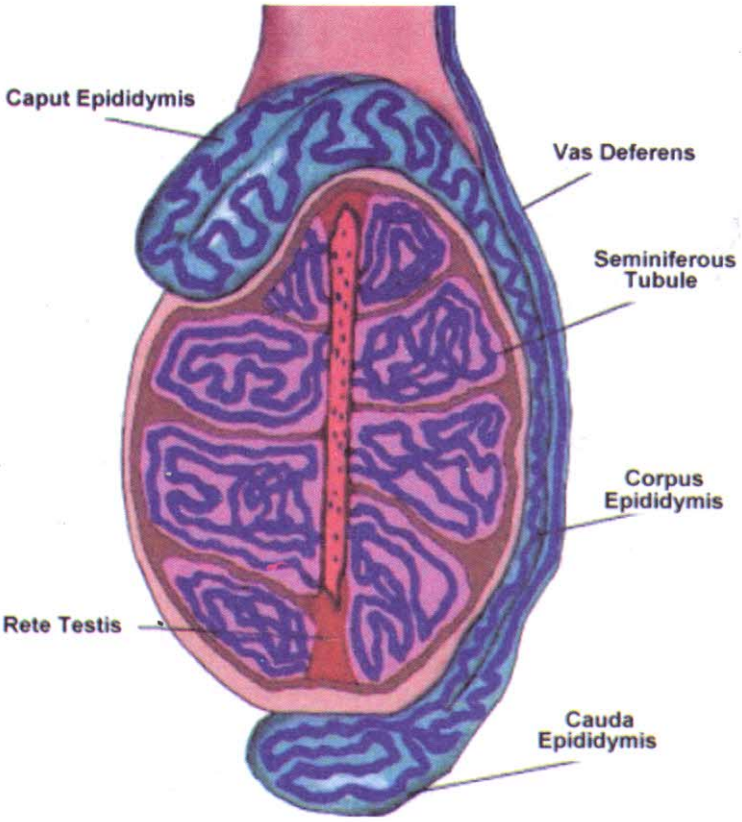


Fig. 3.2 Structure of the testisSource: Geisert 1999

Efferent ductules leave the testis which transport spermatozoa into epididymis, which then continues as ductus deferens. Ductus deferens receives secretions from the accessory glands and joins the urethra.

Size of testis as percentage of body weight is highest in ram (0.4%, 400 g) followed by boar (0.16%, 500 g), stallion (0.06%, 250 g) and bull (0.05%, 400 g). These value fall close to the line relating log testis mass to log body mass. Testicular size varies throughout the year in seasonal breeders (ram, stallion and camel).

Origination of testis occurs during fetal life; the gonads first become apparent and the primordial germ cells migrate to a ridge on one side of the fetal kidney, the mesonephros. By the time the sex of gonad is apparent; the testis appropriates its duct and converts it to epididymis and ductus deferens. At caudal end of the testis a structure called as gubernaculums develops and grows out through the inguinal canal beyond the abdominal wall. Regression of the gubernaculums causes the testis to enter the scrotal cavity where the gubernaculums had been. In most domestic animals the decent of testis is complete at birth while in dogs the decent completes post-natally. Failure of testes to descend is referred to as **cryptorchidism** (condition of hidden testes).

Location of testis is in the scrotum and orientation of the long axis of the testis differs with species. Testicular weight is highly correlated with the scrotal circumference

But some workers have reported that scrotal circumference is unlikely to an accurate predictor of sperm output in AI bulls.

Parenchyma of testis consists of seminiferous tubules separated by interstitial tissues, which includes the smaller blood and lymphatic vessels and nerves as well as hormone producing Leydig cells. Fibrous septa divide the parenchyma into lobules of coiled seminiferous tubules. In center of the testis there is a fibrous mediastenum, containing the channels of rete testis through which the spermatozoa and the fluid in which they are suspended, leave the testis. Interstitial tissues lie between the seminiferous tubules and consist of Leydig cells, blood and lymph vessels, nerves and other cells. Leydig cells secrete testosterone under the influence of LH.

Thermoregulation of testis enables the organ to function effectively in the mammal. Testicular temperature remains lower than body temperature. Location of testis outside the abdominal wall permits regulation of the testicular temperature. Temperature receptors in the scrotal skin provoke panting and sweating leading to lowering of temperature of the whole body. Muscular (dartos) component of the scrotal skin enable it to alter the thickness and surface area of the scrotum and vary the closeness of the contact of testis with the body wall. The smooth muscles within the spermatic cords and the tunica albuginea can lower or raise the testis in horse.

In al farm animals the testicular artery is a convoluted structure in the form of a cone, the base of which rests on the cranial or dorsal pole of the testis. These arterial coils are surrounded by pampiniform plexus of testicular vein (Fig. 3.3). The arterial blood is cooled by the venous blood leaving the testis, lowering down the temperature of the testis.

Testicular cells are shown in Fig. 3.4. Leydig cells lie between the seminiferous tubules and produce testosterone (5mg/day in adults). They also produce estradiol, dihydro-testosterone (DHT), dehydroepiandrosterone (DHEA), androstenedione and progesterone. Sertoli cells are present inside the seminiferous tubules and their function is providing support to production of spermatozoa.

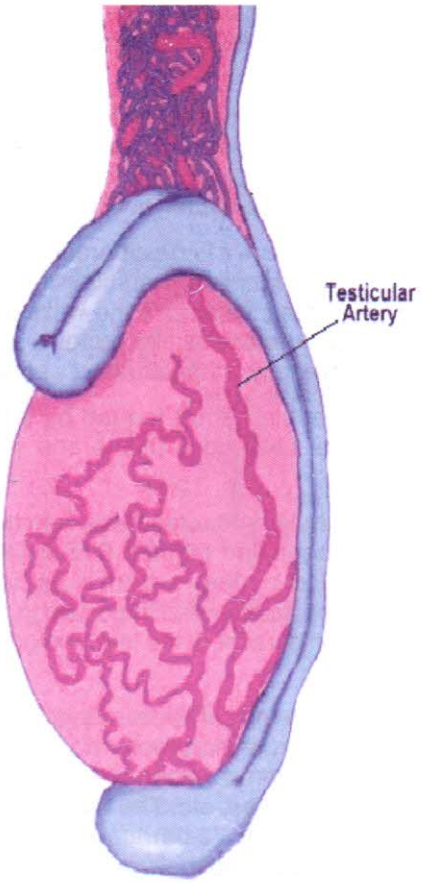


Fig. 3.3 Convoluted testicular artery on the surface of the testis.
Source: Geisert 1999

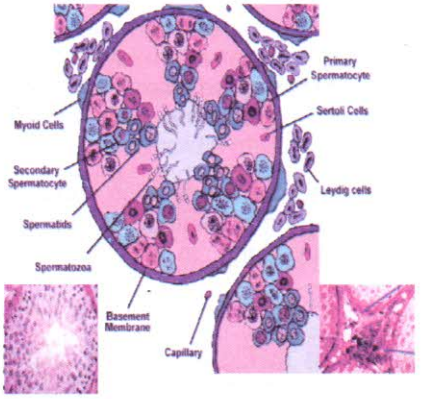


Fig. 3.4 Cross section of the testis Source: Geisert, 1999

They also produce androgen-binding protein intratesticularly, form blood-testes barrier, produce inhibin and produce seminiferous tubule fluid, containing nutrients, causing capacitation and motility of spermatozoa.

Sertolic cells support production of spermatozoa, production of androgen-binding protein intratesticularly (TeBG) and formation of blood-testes barrier. Another function of Sertoli cells is production of inhibin and production of seminiferous tubule fluid (a bathing medium having nutrients supporting capacitation and motility).

Blood testis barrier (BTB) keeps a large difference in composition between tubular and retes testis fluid versus blood plasma and interstitial fluid. The difference is maintained due to restriction movement of substances across the walls of seminiferous tubules. It has been found that large water soluble molecules like proteins are virtually excluded and lipid soluble molecules and water enter readily; other substances like ions enter slowly.

BTB is an almost impermeable barrier formed by the adjacent Sertoli cells in the seminiferous epithelium near the basement membrane also called the seminiferous epithelium barrier, (Fig. 3.5).

Testosterone enters the tubular and retes testis fluid much more quickly than the di-hydro-testosterone. It has been suggested that the BTB is involved in creating conditions appropriate for meiosis, as it is found in many but not in all classes of invertebrate animals and well as in vertebrates studied so far. It is also important in preventing entry of antibodies into the tubules and the segregation of most of the immunologically foreign germ cells in the tubules.

The BTB supports various biological events leading to maturation of spermatozoa after meiosis through creation of microenvironment. It separates the interstitium from the developing spermatozoon and prevents exchange of various biological substances including nutrients, hormones, electrolytes, etc. The barriers of blood with brain, retina and epididymis are composed of endothelial TJ-barrier while the BTB is composed of tight junctions (TJs). The BTB also divides the epithelium into morphological layers of basal and adluminal components. It facilitates the migration of the developing germ cells from the basal to the adluminal regions, across the BTB. This migration occurs at stages VIIVIII of the epithelial cycle in the testis of rats and mice.

The developing germ cells migrate through the remaining epithelium towards the adluminal edge from where they get out at spermiation. The migration through the BTB occurs through a series of events which require changes in the SertoliSertoli and Sertoligerm cell interface, causing movement of the germ cells from the basal to the adluminal compartment.

The cells of the BTB establish communication and coordination at cellcell interface via junctions and a system of signaling mechanism. Little research work is available in this area while much work has been done in exploring the hormonal regulation of spermatogenesis.

Epididymi has been shown in Fig. 3.2, which comes out from the efferent duct at dorsal part of the testis. Epididymis is composed of head called as the caput epididymis, body called as the corpus epididymis and the tail or cauda epididymis. Ductus deferens

leaves the cauda epididymis, supported by a separate fold of peritoneum, readily separable from rest of the peritoneum.

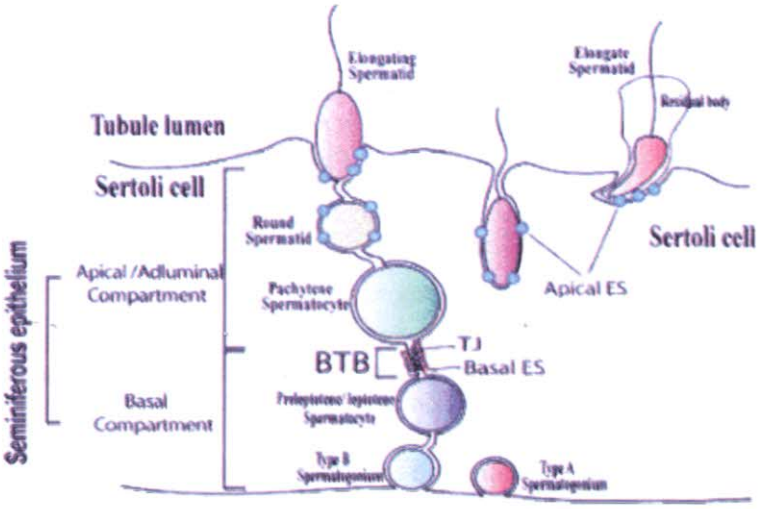


Fig. 3.5. Ultra-structural features of the BTB including the apical ES and the molecular structure of constituent protein complexes at these sites in the adult rat testis. A schematic presentation of the relative position of the BTB and the apical ES in the seminiferous epithelium of adult testis of a rat. The BTB is located between adjacent Sertoli cells in the basal compartment. The BTB sequesters the more developed germ cells (i.e., pachytene spermatocytes and beyond) from the systemic circulation. The epithelium is divided into a basal and adluminal (apical) compartment. Apical ES is found between Sertoli cells and round/elongating/elongate spermatids in the apical compartment (Wong and Cheng, 2005).

Transport of spermatozoa through the epididymis takes about 9-13 days in the farm species facilitated by the flow of the rete fluid, activity of the ciliated epithelium and contraction of the muscular walls of the epididymis. Maturation of spermatozoa takes place during transit through the epididymis and the cytoplasmic remnants on the neck of the spermatozoon migrate to the terminal parts of the mid pieces; motility increases as spermatozoa enter the corpus epididymis. Cauda epididymis is the principal storage organ and spermatozoa retain fertilizing capacity for several weeks.

Functions of epididymis and structure of the cells are controlled by the androgens supplied by the blood stream. Initial segment of the epididymis may be affected by secretions from the rete testis. The androgens slow down the passage of spermatozoa through the epididymis.

Accessory glands contribute their secretion into seminal plasma at the time of ejaculation. Various accessory glands are ampullae, seminal vesicles, prostate and bulbo-urethral (Cowper's) glands (Fig. 3.1). They provide a liquid vehicle for the transport of spermatozoa. Accessory glands work under the influence of androgens regarding their structure and function. Fructose secretion in semen has been used as a useful index of androgenic activity.

Ampullae are the terminal enlargements of the ductus deferens and contribute fructose and citric acid to bull's semen. Seminal vesicles are found on either side of neck of the bladder and were thought to act as store of semen which is no longer true. They contribute to the semen volume and add fructose and citric acid. The prostrate is situated caudal to the seminal vesicles, closer to the junction of the bladder and the urethra. In man and dog the prostrate is well developed, completely surrounding the urethra. Cowper's gland lies near pelvic part of the urethra.

The seminal vesicles of bull are readily detectable on either side of the ampulla and the body of the prostrate gland is detectable as a hard smooth prominence caudal to the neck of the bladder.

Penis and prepuce constitute the external components of the mammalian male reproductive organs. Penis composed of three cavernous bodies around the penile urethra. They are corpus spongisum surrounding the urethra and enlarging at the penile bulb. This bulb is covered by the striated bulbo-spongeosus muscles. The corpus cavernosum arises as a pair of crura from the ischial arch and continues to the apex of penis as a paired dorsal cavernous body. A pair of smooth retractor penis muscle arises from the sacral or coccygeal region of the vertebral column and is especially large in ruminants and swines. These muscles act on the sigmoid flexure increasing length of the penis.

Prepuce is found in ruminants and swine and divided into pre-penile and penile parts. The orifice is controlled by special striated muscle.

Erection and ejaculation are required for semen discharge and it deposition into the female reproductive tract. Erection of penis occurs through dilatation of arteries supplying the cavernous body of the penis. The para-sympathetic fibers in the pelvic nerve are responsible for the vasodilatation. Stiffening and straightening of penis in ruminants is caused by ischio-cavernosus muscle. In bull, ram and goat, there is no veins draining the distal levels of the corpus cavernosum penis and this develops erection pressure in the organ.

Intromission and ejaculation are elicited by tactile stimuli warmth of vagina and the slipperiness of mucus acting on the penile receptors. The penis of bulls and ram are sensitive to temperature and the penis of stallion is sensitive to the pressure exerted by the vagina. The corkscrew shape of penis of the boar is engaged in the cervix during mating which stimulate ejaculation. Electrical stimulation of the sacral nerves causes erection and ejaculation, which is used for semen collection, but the quality of semen, is low.

PUBERTY AND ASSOCIATED FACTORS

Puberty is associated with secretion of sufficient steroids by the gonads coupled with a rapid growth of the reproductive organs and appearance of secondary sexual characteristics. The onset of puberty occurs gradually and it is not a qualitative endocrinological event. This parameter is an important indicator of reproductive efficiency in male and female animals.

Puberty commencement in the bull appears as a result of changes in hormonal profiles which initiate soon after birth and continues with the development of various organs. Average age at puberty of the bull is 9 to 12 months, ranging form 6 to 18 months. During onset of the puberty development of various organs lead to spermatogenesis and semen ejaculates contain spermatozoa, which goes on increasing up to 50 million, with at least 10% having progressive motility.

Changes associated with puberty include a gradual increase in the concentration of LH and testosterone. It is indicative of maturation of the hypothalamo-hypophyseal complex which becomes desensitized to gonadal steroid hormones. The follicle stimulating hormone is maintained at relatively constant levels and episodic peaks are lacking until onset of puberty. In younger bulls the prolactin concentrations increase during age of one through five months, and the frequency of prolactin peaks increase through four months of age. Contribution of prolactin in the process of sexual maturity needs further studies. On one hand there are endocrine changes associated with the onset of puberty and on the other, the seminiferous tubules undergo developmental changes of quantitative and qualitative nature in the testes.

Spermatogenic function is supported by endocrine activity. The spermatogonia are found at eight week, primary spermatocytes at 32 weeks and propagation of spermatocytes at 44 weeks. This time frame of this developmental process may be influenced by breed, environment and nutrition.

In newborn bulls the tube-like seminal vesicles increase in length associated with a double bending. During four to eight weeks the first curvature appears and during 24 weeks the second one. There is a continuous increase in the size of the seminal vesicles up to sexual maturity. In younger bulls reared in groups, the seminal vesiculitis usually occurs, so proper knowledge of the size, shape and consistency of seminal vesicle is needed for examination of younger bulls.

About four weeks after birth penis begins to separate from the prepuce, and during eight weeks the urethral process also separates. At 12 weeks of age the sigmoid flexure starts developing. The process of development can be accelerated through administration of androgen and inadequate nutrition retards it.

After onset of puberty, a bull acquires the capacity of producing at least 50×10^6 spermatozoa/ml; having 10% progressive motility. A relatively constant scrotal circumference of about 27.9 ± 0.2 cm at puberty is found in various breeds although they may differ in biologic type, age and weight at puberty. Lower age at maturity with large testicles is usually associated with daughters showing early maturity and better fertility.

European breeds of bulls reach puberty earlier than Brahman and Brahman based bulls. The first sperm appears in bulls of European breeds earlier than Brahman

bulls and so the appearance of 50 x 10^6 spermatozoa having 10% progressive motility. Brahman and Brahman based bulls achieve reproductive maturity at an older age than European breeds.

Factors influencing puberty include genetics and environment. The *Bos indicus* cattle in the tropics and subtropics reach puberty during 16 to 40 months of age. This age is older than *Bos taurus* x *Bos indicus* crossbreeds or purebred taurine cattle. This variation in age at puberty is caused by genetic and environmental factors, which comprise feeding plan, health status, ambient temperature and season of birth. Growth is effected by these factors.

Relationship of age at puberty has been investigated with production traits through several studies on cattle. It has been reported that age at puberty was associated significantly and negatively with growth parameters and faster growing cattle exhibited a smaller age at puberty. Age at puberty showed stronger genetic correlation with average daily weight gain, weight at puberty and weight at one year of age while the phenotypic correlations was weaker.

Puberty is delayed by poor nutritional status in cattle, significantly. The role of body weight is greater than age, in the onset of puberty. The onset of puberty is affected by physical environment, age, breed of dam and sire and photoperiod. High-protein diet in cattle lead to younger and heavier animals at puberty than the animals on a low-protein diet and such animals show higher fertility during post-pubertal life. Although poor nutrition results in delayed puberty, a very high feeding plan does not necessarily result in earlier onset of puberty.

Existence of parasite burden has resulted in poor reproductive performance in cattle in Australia. The experimental cattle were grazed in four groups: a) control without any treatment for parasitic infestation; b) monthly treatment with acaricide against ecto-parasites; c) monthly treatment monthly with anthelminthics for endo-parasites; and d) monthly treatment with treatment for ecto and endoparasites. After 25 months, 197 of 212 heifers exhibited symptoms of puberty. A significant difference was noted in weight at puberty among groups but age showed no effect. The breed and treatment interaction did not affect either age or weight at puberty.

Buffalo bulls reach sexual maturity at 2 to 3 years of age. Semen is produced all year round but it is highly affected by heat stress and low quality feed. The buffalo bull seems to be most fertile in spring when the volume of ejaculate and the sperm concentration is the highest. The vitality of the sperms is also much higher in spring than other times of the year. Corresponding values are lowest in summer time. Heat stress may have a negative effect on libido.

Table 3.1 Age at Puberty (months)

Species	Male	Female
A. Farm animals		
Cattle	11 (7-18)	11(9-24)
Sheep	7 (6-9)	7(4-14)
Goats	7-8 (4-12)6-8	
Swine	7(3-8)	6 (3-7)
Horses	14(10-14)	18(12-19)
B. Age at Puberty (Other Mammals)		
Rabbits	3 to 4 months	
Cat	8 to 9 months	
Dog	10-12 months	
Humans	10-16 years	

Hypothalamus (Tonic Centre)

- Gonadotropin pulsatility increases testosterone
- Testosterone initiates puberty

Hypothalamus (Pre-ovulatory Centre)

- Absent in male
- Fetal testosterone defeminizes hypothalamus
- Eliminates GnRH surge center
- Mechanism: Testicular testosterone enters brain
- Converted to estradiol (aromatization)
- Suppress surge center development

In female no defemination of hypothalamus as:

- Estradiol bound to alpha fetal protein (AFP)
- Complex can't cross Blood-Brain Barrier

Fig. 3.6 Endocrine mechanism of onset of puberty through male hypothalamus (Source: Winters, 1998)

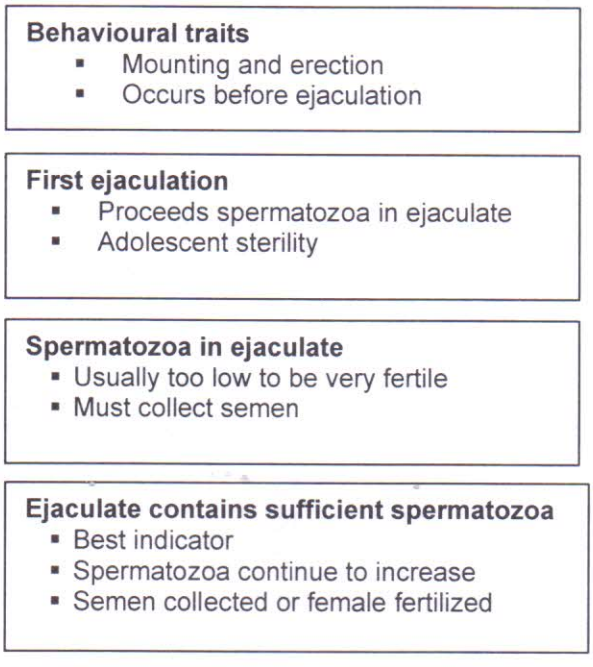


Fig. 3.7 Criteria for onset of puberty in males (Winters, 1998)

SPERMATOGENESIS, SPERM MATURATION AND STORAGE

Spermatogenesis is the production of male gametes or spermatozoa. It occurs in the seminiferous tubules which contribute to about 90% of the testicular mass. The seminiferous tubules show gradual changes in histology, with age. There is a considerable difference in time required for spermatogenesis vs. oogenesis i.e., about 9-10 weeks vs. 10-50 years. The production of mature spermatozoa (haploid, 1*n*) from spermatogonia (diploid, 2*n*) is essential for the perpetuation of all mammalian species.

Although spermatogenesis varies in detail in different species (e.g., minks are seasonal breeders exhibiting seasonally or environmentally responsive phases in this process whereas spermatogenesis continues throughout the entire life span in humans and rodents), the cellular constituents and the basic physiology of the testes are rather similar. Rats, mice and men have been used extensively for studying spermatogenesis than other species.

SPERMATOGENESIS

The spermatogenesis is initiated at the level of primordial germ cells which are formed during embryonic development; and migrate from the yolk sac region of the embryo into the undifferentiated gonads. These cells divide several times before becoming gonocytes. Just before puberty the gonocytes type A1 spermatogonia form A2, A3 and A4 spermatogonia. A4 spermatogonia divide to form type In (intermediate) spermatogonia and then type B spermatogonia. These cell types may be seen in

histological preparations of the seminiferous tubules, with the whole number (2n) of chromosomes. Spermatogenesis is graphically presented in Fig. 3.8. Semen producing capacity of various farm animals is given in table 3.2 and semen characteristics in table 3.3.

Type B spermatogonia (2n) divide forming the primary spermatocyte (2n). The primary spermatocyte duplicate their DNA and undergo progressive nuclear changes of meiotic prophase known as preleptotene, leptotene, zygotene, pachytene and diplotene before dividing to form secondary spermatocytes. The resulting secondary spermatocytes divide again to form haploid cells known as spermatid.

Small cytoplasmic or intercellular bridges are retained between most of the dividing cells which are important for coordination to lead into simultaneous cell divisions. The primary spermatocyte undergoes first meiosis giving rise to two secondary spermatocytes which in turn undergoes second meiosis forming four spermatids with half number of chromosomes (n). The spermatids differentiate (spermiogenesis) forming four sperm cells (n).

SPERMIOGENESIS

Several morphological changes occur in the shape of spermatid to form the spermatozoon, which are collectively known as spermiogenesis. These changes are shown in Fig. 3.9. Condensation of the nuclear chromatin, formation of sperm tail or flagellar apparatus and development of the acrosomal cap are the main changes. Four phases have been classified in the development of spermatozoon form the spermatid. These are Golgi phase, cap phase, acrosomal phase and maturation phase.

The Golgi phase is characterized by formation of PAS (periodic acid Schiff) positive pro-acrosomal granules within the Golgi apparatus. These granules convert into a single acrosomal granule adhering to the nuclear envelope and formation of tail on the opposite pole.

During the cap phase the adhering acrosomal granule spreads over the surface of spermatid nucleus. The process continues until nearly two third of the anterior portion of the nucleus is covered by a thin double layered membrane sac that closely adhere to the nuclear envelope. The distal centriol gives rise to the tail at the opposite pole of the acrosome and elongates well beyond periphery of the cellular cytoplasm.

During acrosomal phase, major changes occur in the nuclei, the acrosome and the tail. The chromatins are condensed and the spheroidal nucleus reshapes into an elongated flattened structure. The acrosome also condenses and elongates to correspond to the shape of nucleus. These changes are molded by the Sertoli cells. Middle piece of the tail is formed by concentration of the mitochondria close posterior to the acrosome.

MATURATION OF SPERMATOZOON

The chromatin granules in the nucleus of the spermatid condense until they form fine homogeneous material that uniformly fills the entire nucleus. A fibrous sheath and the underlying nine coarse fibers are formed around the axoneme. These continue with columns in the neck of the connecting piece of the spermatid. The mitochondria become tightly packed into continuous sheath extending from neck to the annulus. The remaining

cytoplasm is converted into spheroidal lobule called residual body. It completes the process of spermiogenesis and maturation stages and the resulting spermatozoon is ready to release into the lumen of the seminiferous tubule.

SPERM MIGRATION AND STORAGE

The sperm cells migrate to epididymis, where they acquire motility. Sperm is added with fluids, sugar, enzymes, mucus, amino acids, etc. to form seminal plasma. Several hundred million sperm are produced per day in various mammals. Various characteristics of semen are given in various mammals in table 3.2.

The duct wall of the epididymis contracts locally and assists in transportation of spermatozoa. In rat epididymis these contractions have been demonstrated at a rate of 3 per minute and can be stimulated by prostaglandins. Transportation completes during 7 days in a bull and may be accelerated by 10 to 20% by increased frequency of ejaculation.

During transportation through the epididymis, the sperm acquires the capacity of progressive motility reflecting both qualitative and quantitative changes in the metabolic pattern of the flagellar apparatus. This capacity enhances with the advance movements of spermatozoa further. Epithelial cells linings of the epididymis secrete immobilin in some laboratory animals and quiescence factor in the bull probably prolonging the survival by preventing unnecessary metabolism. During the migration the proximal droplet migrates from the neck to the position near the annulus.

Initial capacitation of spermatozoa (attainment of the ability to fertilize and ovum) occurs during their transportation through the epididymis. This capacitation is associated with the motility and especially the progressive motility. The capacitation increases with the advanced movement of the spermatozoa within the epididymis.

Cauda epididymis is the major site of storage of spermatozoa in male reproductive system. Tail of the epididymis contains 70% of the total spermatozoa. However, this storage can not be maintained for an infinite period and are gradually excreted into urine. The spermatozoa not excreted into urine after a prolonged storage, undergo senescence and disintegration. Semen collected after a prolonged sexual rate contains higher percentage of degenerating and dead spermatozoa.

HORMONAL REGULATION

Hormonal regulation of spermatogenesis is controlled by the hypothalamus producing GnRH (Gonadotropin-releasing Hormone) which acts upon the anterior pituitary that produces LH and FSH. LH acts upon interstitial cells (Leydig cells) in testes that produce testosterone. Testosterone and FSH jointly influence the Sertoli cells and spermatogonia to facilitate sperm formation.

Testosterone causes a negative feedback on hypothalamus and pituitary reducing the GnRH, LH and FSH production. Testosterone also modulates development of secondary sexual traits (facial hair, breasts, etc) and sexual (and social) behavior. This is also called testosterone poisoning. Sexual intercourse is a component of social behaviour.

QURANIC WISDOM

Semen has been mentioned by the Quran as "*Nutfa*" meaning "very little water" or "a drop of water", in Arabic. This is similar to the semen ejaculate containing spermatozoa, the male gametes. The shape of spermatozoon is like a fish with long tail which is a meaning of another Quranic word *Sulalah*. Allah the Almighty mentions in Quran; *"He It is Who created all things in the best way and began the creation of man from clay, and made his progeny from an extract of despised fluid (Sulalah)"* (32: 7-8).

"Extract" is another meaning of *Sulalah* which is used for the essential or part of a mass. In light of these descriptions the semen is mentioned as a fluid responsible for creation of man and other mammals. Creation of mammal is further clarified by mentioning spermatozoon with is a part of the semen ejaculate.

The role of the *nutfa* in creation, has been clarified by Allah the Almighty, saying; ***"So, let man consider of what he was created. He was created of gushing water"*** (86: 5-6). At another point it has been mentioned that; ***"He has created man from a sperm (fluid-drop) and behold this same (man) becomes an open disputer."*** (16: 4).

According to the Quran man has not been created from the whole semen, but only a small portion of it. The modern science has only recently known the fact that mammalian life originates from spermatozoon which is only part of the semen and not the whole mass of semen. However, the Quran has mentioned this fact much earlier, before the existence of any scientific information, which shows the divine origin of the Book.

Quran reminds man of his origin from the genital fluids of man and woman, possessing all the hereditary characteristics. It has been said, ***"Woe to man! What has made him reject (Allah)? From what stuff has He created him? From a nutfa (fluid-drop) He has created him, and then moulds him in due proportions."*** (80: 17-19).

The Quran states, ***"Verily We created man of a fluid-drop (nutfa), mingling (amshaj), in order to try him: so We gave him (the gifts of) hearing and sight."*** (76:2). Two words are used in the Quran for seme, i.e., Nutfa and Amshaj. Nutfa is used for a drop of water and *amshaj* is structure consisting of various substances. These statements reveal the miraculous nature of the Quran. This verse rightly describes the nature of semen which, on one hand is a drop of water and on the other it is a mixture of numerous substances comprising spermatozoa, nutrients, enzymes, hormones and other factors.

Sex of mammal progeny is determined by the male or female sex chromosome bearing spermatozoa. X bearing spermatozoon will give rise to female young one if it fertilizes an ovum. An Y bearing spermatozoon will lead to birth of a male young one. According to the Quranic verse; ***"That He created the pairs, male and female, from a fluid-drop sperm as it is emitted."*** (53: 45-46), which confirms that man's sex is determined at the time of semen ejaculation. The Prophet Mohammed told the people that the sperm *(nutfa)* was responsible for determining the sex of the young one (through X or Y bearing spermatozoa).

The scientists have discovered very recently that sex of the embryo is determined by the type of spermatozoon fertilizing the ovum and sperm is a cell found in the ejaculated semen. On one hand the whole world was not aware of the fact the semen

determines the sex of the young one till the beginning of the 20th century. On the other the Quran narrated this story very clearly 14 centuries earlier.

Spermatogenesis takes place in the testicles, which is a continuation of gonocytes, originating near the kidneys, located at the back and then migrating down to the abdominal cavity at the end pregnancy. The Quran has stated earlier: ***"And remember when your Lord brought forth from the children of Adam, from their loins, their seeds..."*** (7: 172). The origin of progeny has been at the region of the back which is the site of formation of embryonic testicles.

Table 3.2. Sperm Producing Capacity of testis in various farm animals

Sperm	Bull	Boar	Ram	Stallion
x 10^6 /gram of testis/day	16	27	25	20
Testis wt. (grams)	350	360	275	200
Total sperm production. (x 10^9 sperm)	11	19	14	8
Spermatogenesis (Days)	61	34	49	49

Source: Geisert (1999)

Table 3.3 Semen Characteristics in Various Farm Animals.

Semen Characteristic	Bull	Ram	Boar	Stallion	Cock
Ejaculate Volume (ml)	5-8	0.8-1.2	150-200	60-100	0.2-0.5
Sperm concentration (million/ml)	800-2000	2000-3000	200-300	150-300	3000-7000
Sperm/ejaculate (billion)	5-15	1.6-3.6	30-60	5-15	0.6-3.5
Motile sperm (%)	40-75	60-80	50-80	40-75	60-80
Morphologically normal sperm (%)	65-95	80-95	70-90	60-90	85-90
Protein (g/100 ml)	608	5.0	3.7	1.0	1.8-2.8
pH	6.4-7.8	5.9-7.3	7.3-7.8	7.2-7.8	7.2-7.6
Fructose	460-600	250	9	2	4
Sorbitol	10-140	26-170	6-18	20-60	0-10
Citric acid	620-806	110-260	173	8-53	0
Inositol	25-46	7-14	380-630	20-47	16-20
Glyceryl phosphoryl choline	100-500	1100-2100	110-240	40-100	0-40
Ergothioneine	0	0	17	40-110	0-40
Sodium	225	178	587	257	352
Potassium	155	89	197	103	61
Calcium	40	6	6	26	10
Magnesium	8	6	5-14	9	14
Chloride	174-320	86	260-430	448	147

Hafez (1987)

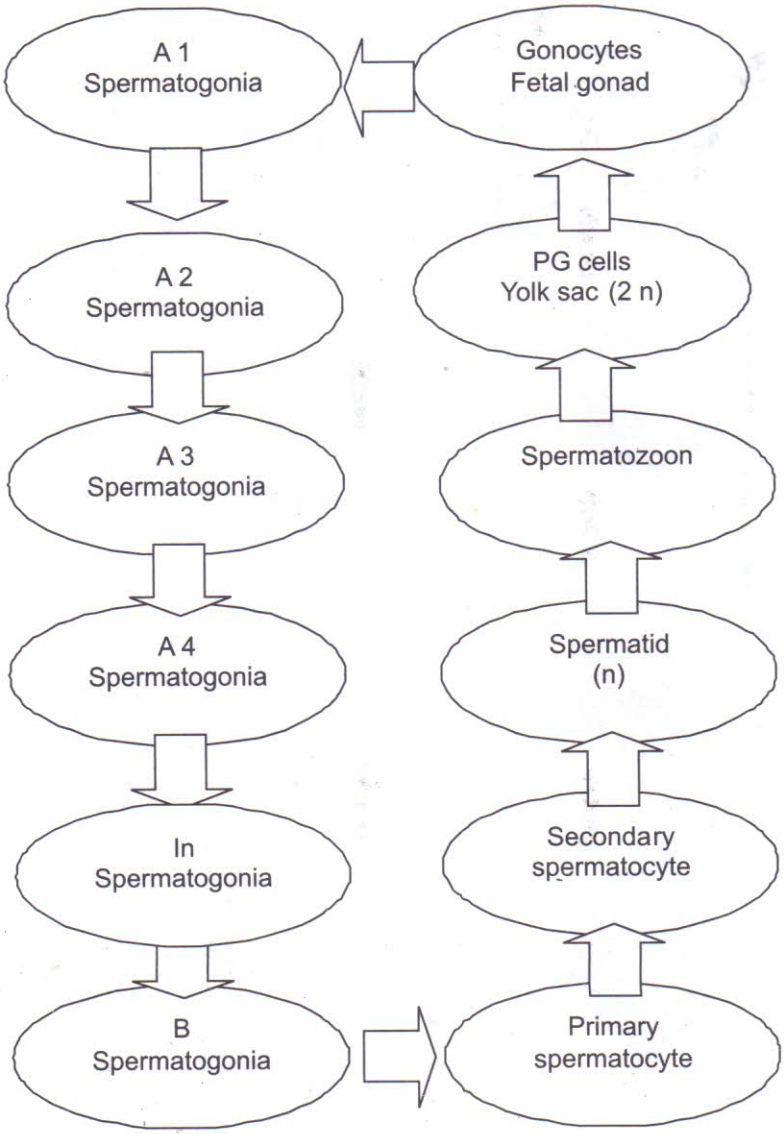
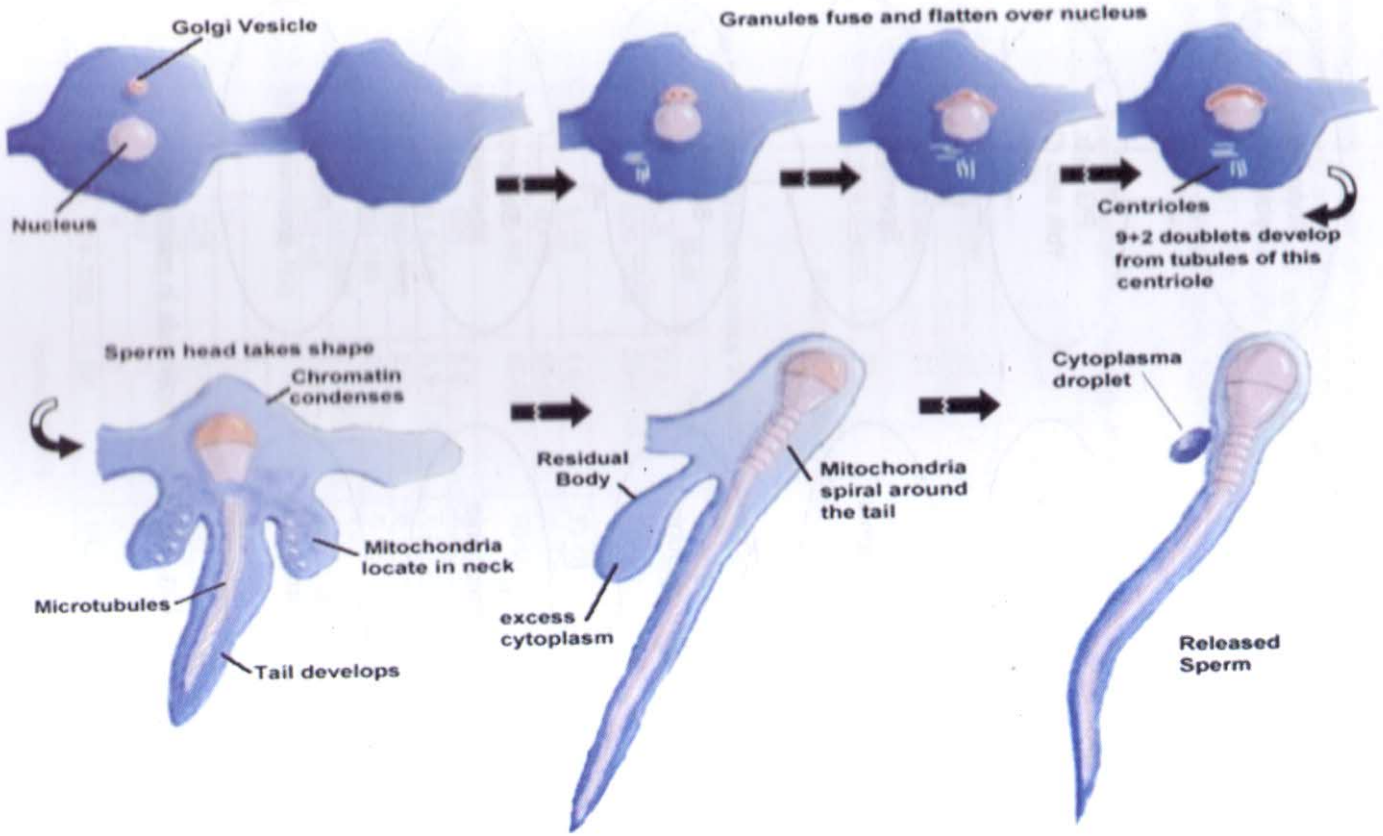


Fig. 3.8 Spermatogenesis in mammals


Golgi Vesicle
Nucleus
Granules fuse and flatten over nucleus
Centrioles
9+2 doublets develop from tubules of this centriole
Sperm head takes shape
Chromatin condenses
Mitochondria locate in neck
Microtubules
Tail develops
Residual Body
excess cytoplasm
Mitochondria spiral around the tail
Cytoplasma droplet
Released Sperm

MATING AND SEMEN EJACULATION

Mating has been described under the heading sexual behavior in the second chapter on female reproductive physiology. It is the second component of sexual behavior after courtship and is manifested by immobile standing of female, mounting of female and ejaculation. ***Intromission and ejaculation*** are elicited by tactile stimuli warmth of vagina and the slipperiness of mucus acting on the penile receptors. The penis of bulls and ram are sensitive to temperature and the penis of stallion is sensitive to the pressure exerted by the vagina. The corkscrew shape of penis of the boar is engaged in the cervix during mating which stimulate ejaculation. Electrical stimulation of the sacral nerves causes erection and ejaculation, which is used for semen collection, but the quality of semen, is poor.

SEMEN COMPOSITION

Semen is the male ejaculate, the suspension consisting of spermatozoa suspended in seminal plasma. Cellular portion of the semen are the spermatozoa, known as the male gametes which are produced by the seminiferous tubules of the testis. Fluid portion of the semen, the seminal plasma, comprise of secretions from the epididymis and accessory glands of the male reproductive tract. **Table 3.2 reports the semen characteristics in various mammals.**

Chemical components of spermatozoon are nucleic acids, proteins and lipids. One third of the weight of nucleus is contributed by the nucleus. The inorganic constitutents are phorphorus, nitrogen and sulpher. The sulfhydryl bonding which occurs in spermatozoon increases as the cell passes through the epididymis. This bonding increases condensation and stabilization of the sperm DNA.

Composition of seminal plasma varies with the species as does the size and number of accessory glands. Seminal plasma originates from the testis, epididymis and the accessory glands. The only accessory gland common to all species is the prostate gland. The seminal plasma contains unusually higher levels of citric acid, ergothioneine, fructose, glycreylphosphorylcholine and sorbitol. Appreciable quantities of ascorbic acid, amino acids, peptides proteins, lipids, fatty acids and enzymes are also present in seminal plasma. Antimicrobial constituents of seminal plasma are seminal plasmin and immunoglobulins, mainly of the A class. Prostaglandins, androgens and estrogens, FSH and LH are also found in seminal plasma.

Concentration of various constituents of seminal plasma depends upon the rate of testosterone secretion by the testis, and varies among different animals of the same species, in different samples of semen from the same animal and in successive fractions of the same ejaculate. For example in stallion the pre-sperm fraction contains lower citric acid and ergothioneine levels as compared to the sperm rich fraction.

SPERM MORPHOLOGY

The spermatozoon is composed of the head, the neck and the tail. The fully formed spermatozoa are elongated cells consisting of flattened head, containing the nucleus, and a tail containing the apparatus for cell motility. The whole sperm cell is

covered by a membrane known as the plasmalemma or plasma membrane. A short neck connects the sperm head with the tail. Sperm structure is given in Fig. 3.9. Sperm length is species-specific and varies in well-known farm and companion animals between 50 µm (boar) and 90 µm (bull) (Table 3.3 and Fig. 3.9, 3.10, 3.11).

The sperm head is an oval, flattened nucleus containing highly compact chromatin. The condensed chromatin is composed of deoxyribonucleic acid complexed to a special class of basic proteins known as sperm histones. The number of chromosome in the sperm nucleus is half of the somatic cells of the species, called as haploid. Haploid nature of the sperm chromosomes is due to meiotic cell division of the secondary spermatocytes.

The acrosome covers the anterior end of the sperm nucleus, and is a thin double layered membrane. Acrosome is a cap like structure, closely applied to nucleus during the last stages of spermatogenesis. Various hydrolytic substances are present in the acrosome which are enzymes in nature and include acrosin, hydrolases, hyaluronidaze and estrases. These substances help in the process of fertiiization.

The acrosome is constituted by an inner and an outer membrane which merges in the posterior region of the acrosome. The acrosome encloses the fine granulated, electron dense contents of the nucleus of spermatozoon. It comprises of three components: i) the apical or anterior margin; ii) the main segment that backs over the anterior half and; iii) the equatorial segment or caudal portion of the cap. The main and equatorial segment joins together through an abrupt decrease of thickness of the cap and an increase in density. The acrosome and nucleus are separated by a sub-acrosomal space having amorphous contents of low density. This space provides binding between the nucleus and the cap of the spermatozoon.

The nucleus contains genetic materials called chromatin which carry genetic information. The nucleus varies in shape in different species and is usually flattened dorso-ventrally. Transmission electron microscopy (TEM) is used for studying the sperm nucleus and it appears very dense due to the highly condensed chromatin. An envelope comprises the nuclear membrane and an acrosomal membrane. At the caudal end of the nucleus there is a concave implantation fossa which is used for attachment of the sperm head to the connecting piece called as capitulum.

The sperm tail is attached with the head posterior to the acrosome. It comprises of the neck, the mid piece, the principal, and the end piece. Axoneme is the name given to the core of the middle piece and the entire length of the tail. It is comprised of nine pairs of microtubules that are arranged radially around two central filaments. The axoneme and associated dense fibers of the middle piece are covered peripherally by numerous mitochondria. The axoneme is responsible for sperm motility. The outer pairs of the microtubules generate the bending waves of the tail.

The neck is attached to the basal plate anteriorly and to the outer dense fibers of the flagellum, posteriorly. Size of the neck is about 1 µm. The neck varies in length due to the changes in size and shape of capitulum. The neck is a segment like structure linking the flagellum with the sperm head. The capitulum is a dense fibrous structure, next to which lies the proximal centriole, however, and the distal centriole can not be seen in the mature spermatozoa. Around the centriole fibrous proteins are found in the early

spermatid and later they form two major and five minor columns. Each one of the major columns divide further into two making finally, a total of nine columns with similar diameter which are followed by nine outer dense fibers of the flagellum (ODF) at the junction of neck and middle piece. Single mitochondria lies between the longitudinal columns, with smaller projections into the connecting piece. Mitochondria supplies energy for sperm movements and the neck provides an articular piece for connection.

The mid-piece is about 5 µm in length and is possesses the mitochondrial sheath which overlaps the axonemal complex along with the nine ODF. The sperm tail is different from simple flagella or cilia by having ODF and fibrous sheaths (FS) which provide cytoskeletal elements in addition to the central axoneme. ODF are found in species which perform internal fertilization. Survivability of spermatozoa and the number of mitochondria are correlated. Spermatozoa of mammals having many mitochondria posses higher survivability as compared to spermatozoa of aquatic vertebrates, having two mitochondria. The Jensen's ring is located at the caudal end of mitochondrial sheath also known as the annulus and it provides a barrier separating midpiece from the principal piece. Flagellar membrane is attached to the annulus. The annulus helps in the tail movements and prevents a displacement of mitochondria on caudal side.

The principal piece is longest of all segments and is contained in fibrous sheaths. The tail is made of two longitudinal columns on dorsal and ventral side. The ribs are oriented circumferentially around the tail. The ribs get thickened while crossing over to the columns. In some species broad bands are formed through interconnection of each other. The columns seem to less closely packed as compared to subunits of the ribs, if examined at higher magnification. The ribs of the fibrous sheaths run independently but at their ends, they sometimes bifurcate. The fibrous sheath ends 910 µm shorter than the tip of tail and the principal piece links with the end piece. This helps in preventing caudal displacement when the contraction waves during sperm motility continue. In addition, the fibrous sheaths help in maturation of spermatozoa and motility. Hyperactivation and capacitation are also assisted.

The end piece is located at the end of the tail and the 9 × 2 + 2 arrangement cease to exist. The 9 doublets are converted to 18 single microtubules. Most of the structures like the fibrous sheaths, ODF and even the central tubule pair may not be seen there.

MOTILITY AND ABNORMALITIES OF SPERMATOZOA

Motility is required for stimulation of fertilization. The mammalian sperm is pushed forward by the propulsive forces of flagella which facilitate the sperm to reach the fertilization site located in the oviduct. Motility also enables the sperm to penetrate the ovum. The cyto-skeletal compounds help in sperm motility through flagellar motion. Energy is provided by the mitochondria in the form of ATP which is consumed for motility. A motor protein "Dynein" is responsible for intracellular transport of substances. This protein has been found to play a significant role in organization and motility of flagella. Chemical energy (ATP) is translated into kinetic one through the interaction of ATP and ATPase on the dynein arms. This causes sliding of the adjacent microtubule doublets relative to one another. Absence of the dynein arms will cause immotility of the spermatozoa. This causes "immotile-cilia syndrome" in man and has been found .

as an inborn error.

Morphological abnormalities in sperm structure are usually seen in semen samples and constitute a major cause of infertility. Abnormalities may be categorized as: i) primary, when the sperm head and acrosome are involved; ii) secondary, when droplets are present on the mid-piece of the tail and; iii) tertiary when other tail defects are involved. Figure 3.12 shows abnormalities in Eosin-Nigrosin stained spermatozoa of various cattle breeds. In the semen of bull, when the abnormal sperm cells increase 20%, it reduces fertility.

Heat stress causes abnormalities in spermatozoa under high ambient temperature coupled with high humidity. It reduces a male's fertility which may remain for up to six weeks, producing large number of abnormal sperm. Before the onset of puberty, the bull semen possess higher incidence of primary abnormalities, mostly the proximal droplets. However, a significant decreased has been observed with the advancing age. Percentage of spermatozoa with proximal droplets diminishes significantly as the animal ages. It has been concluded that abnormality of spermatozoa decreased the rate of fertilization and embryo development. The occurrence of proximal droplet in young bulls decreases with the increasing age.

Scrotal circumference has been found as a good indicator of normal semen and a circumference measuring below the average for their age does not support production of normal semen than their normal counterparts. Pre-pubertal beef bulls exhibit low sperm count and motility, besides a high number of abnormal spermatozoa. It has been reported for *Bos taurus* bulls, that 52.1 and 12.5% of animals were rejected due to high number of abnormal sperm/insufficient scrotal circumference.

Table 3.4. Measurements of various parts of spermatozoa in farm animals (µm)

Species	Head		Mid-piece		Principal piece	
	Length	Width	Length	Width	Length	Width
Man	3.4–4.6	1.5–2.8	3.5–5.0	0.8–0.6	44–50	0.5–0.4
Stallion	5.0	2.4	8.0	0.5	30	0.49
Bull	9.15	4.25	14.84	0.67	50	0.51
Boar	8.5	4.25	10.0	–	30	--
Sheep ram	8.2	4.25	14.0	0.8	45	0.5

(Pesch and Bergmann, 2006)

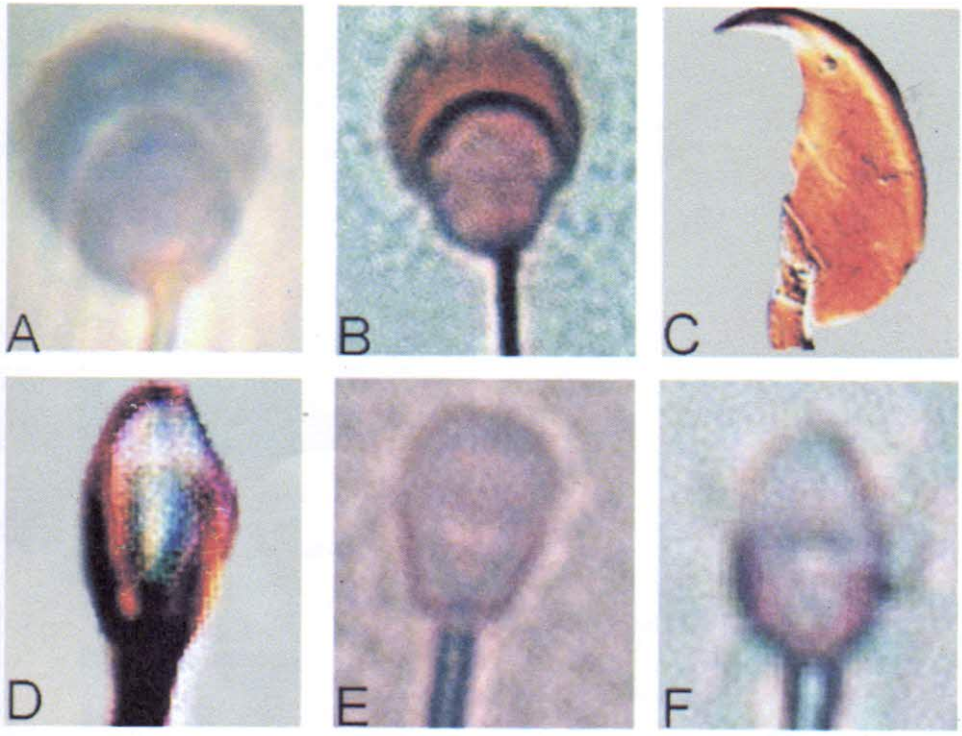


Fig. 3.10 Sperm heads of different mammals in light microscopy (magnification: 400×), (A) guinea pig, (B) rabbit, (C) mouse, (D) stallion, (E) dog, (F) man. (Pesch and Bergmann, 2006)

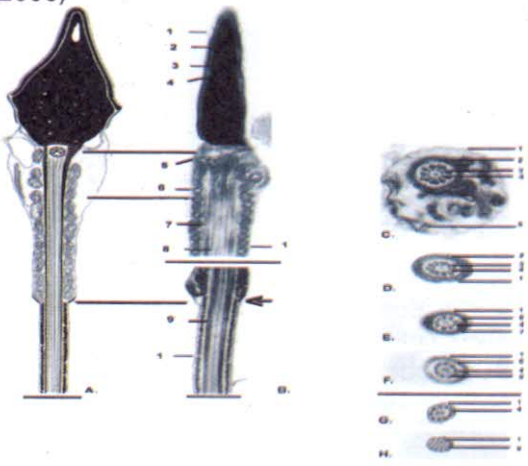


Fig. 3.11. (A) Human sperm (B) Stallion sperm (C)–(H) cross sections (C) proximal midpiece, (D) distal midpiece (E)/(F) principal piece, (G)/(H) end piece (1) Plasma membrane, (2) acrosome, (3) nucleus, (4) small nuclear vacuole, (5) proximal centriole, (6) mitochondria, (7) outer dense fibres (ODF), (8) outer tubule doublet, (9) fibrous sheath (Pesch and Bergmann, 2006)

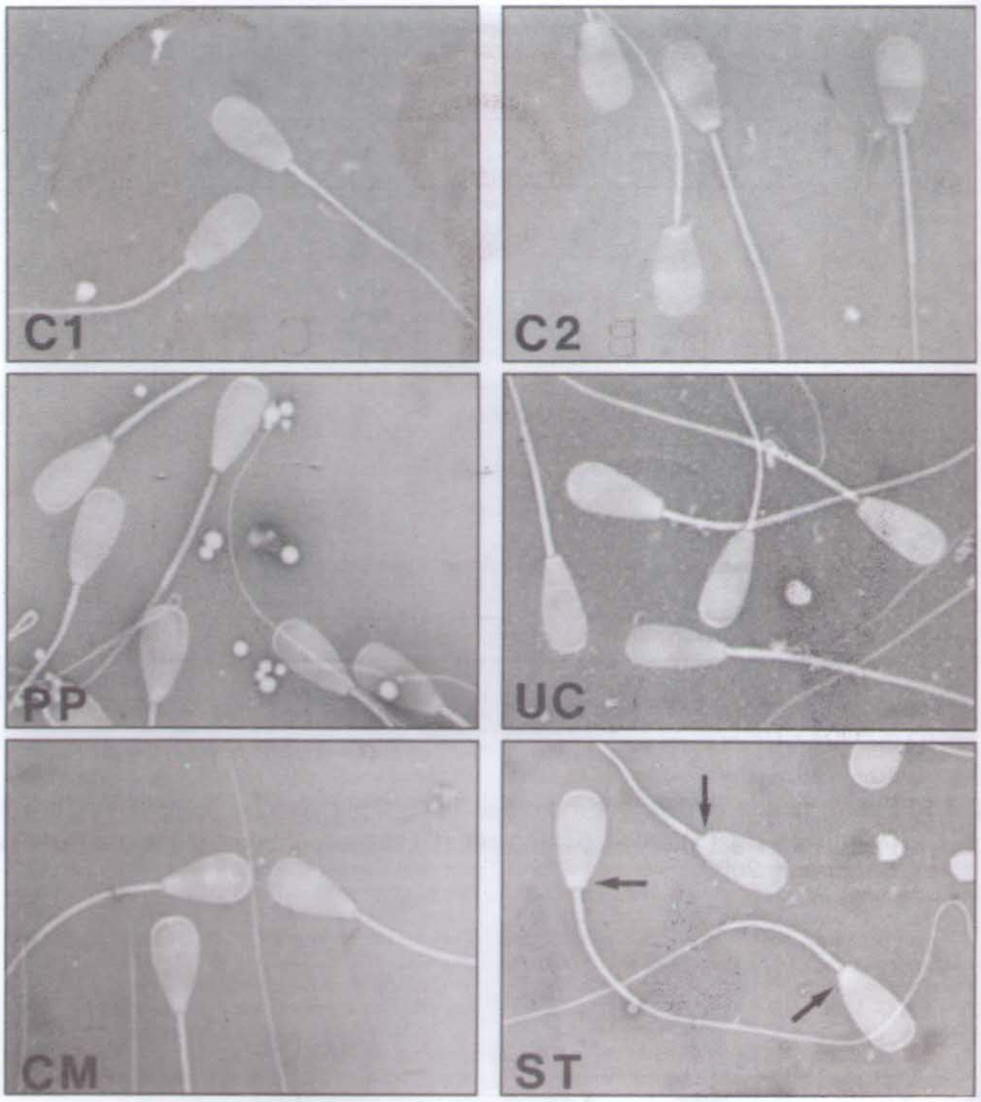


Figure 3.12 Abnormalities in Eosin-Nigrosin stained spermatozoa. C1: Control semen Ayreshire; C2: Control semen Simmental; PP: Moderately pyriform heads of Hosltein; UC: Moderately tapered heads of Limousin; CM: Moderately pyriform heads of Texas longhorn; ST: Sperm heads pinched at the base. (Barth et al., 1992)

FACTORS INFLUENCING SEMEN QUALITY AND VOLUME

Normal semen is produced by a normal male and is a milky suspension of sperm cells. Table 3.3 has reported characteristics of normal semen in various farm animals. The quality and volume of semen is affected by various factors. Based on semen parameters, the breeding male animals have been classified into satisfactory, questionable and unsatisfactory on the basis of breeding potential.

Semen quality is evaluated on the basis of color, volume, motility (gross and progressive) and morphology. The parameters are used for semen evaluation under routine practices. Many parameters have been used in selecting a sire for natural service or cryopreservation of semen, however certain crucial parameters like semen motility and morphology have been found as more useful indicators of semen quality. In fact, the final goal of semen evaluation will be to predict the fertilizing capacity of a semen ejaculate.

Semen volume, along with other characteristics of semen has been given in Table 3.2. The ejaculate volume in various species have been reported to be 5-8 ml in bull, 0.8-1.2 ml in ram, 150-200 ml in boar, 60-100 ml in stallion and 0.2-0.5 ml in cock.

It has been found that traditional characteristics of spermatozoa influence fertility up to a limited extent and the variability in results is much higher. Therefore, prediction of fertilizing capacity has been a great challenge to the veterinarians, as no single parameter can predicts fertility in vivo in an accurate manner. Therefore, advanced techniques are required for evaluation of semen to assess the fertilization capacity of semen.

Factors affecting semen quality and volume mainly relate to environment as the livestock population is found in various climatic zones of the world. Extensive investigations have been made to study the environmental effects related to different climatic conditions in different breeds and various regions. It has been reported that season was an important factor influencing semen volume and quality.

Seasonal effects of semen quality comprise various factors like temperature, humidity, photoperiod, nutritional status and management. Season effects semen production but the reports are contradictory to each other. Spring and winter have been found favorable season for semen volume and quality by many studies. An ambient temperature of 15–20 °C has been found as the best one, for producing good quality semen.

The ambient temperature on the day of semen collection, and also that during maturation or spermatogenesis in the testis during about 70 days before semen collection probably affects semen production. Different bulls respond to temperature differently indicating a difference among the bulls regarding the heat tolerance. Nutritional status of the bulls affects semen quality during the process of spermatogenesis.

The semen collection attendants and technicians take care of sexual preparation of the bulls before semen collection which is important for semen quality. The team affects semen quality regarding volume and total number of spermatozoa while sperm concentration and motility are not effected.

Semen collection interval and frequency influence the quality of semen. First ejaculate provides significantly larger volume of semen volumes along-with sperm concentration and total number of spermatozoa; however no affect is observed on motility. Similarly, semen production is also influenced by weekday.

Age of bulls exerts a highly significant effect on ejaculate characteristics as reported by many studies. As a general principal the increasing age of bulls results in an increasing semen production capacity and quality. However, the effect of management and health may confound the effect of age misleading the results. Good quality semen may be obtained from some older bulls through preferential treatment while a poor health status may deteriorate semen quality and volume.

SEMEN TRANSPORTATION IN THE FEMALE REPRODUCTIVE TRACT

During sexual intercourse or artificial insemination, the spermatozoa are deposited in the vagina. The ova released from the ovary and picked up by the infundibulum, travels through oviduct and reach the site of fertilization. Survival of the ova and the spermatozoa is only for a shorter period of 20 to 48 hours, so the gametes must reach the site of fertilization, the isthmus-ampullary junction. The two types of gametes travel simultaneously at opposite direction to reach the fertilization site.

Factors facilitating the gametes transportation are the inherent contractility of the female reproductive tract, regulated by the central nervous system and hormonal activity. Certain active substances in the semen stimulate and modulate the contractility of the female reproductive tract. The oviductal cilia and fluid also facilitate gametes transportation.

After insemination the spermatozoa penetrate the micelles of the cervical mucus where some are quickly transported through the cervical canal. This phase lasts for 2-10 minutes and may be facilitated by the movement of spermatozoa and the increased contractile activity of the uterus and the oviducts.

Sperm reservoirs are the complex mucosal fold of the cervical crypts. The micelles of the cervical mucus help to direct spermatozoa to the cervical crypts where the reservoir is formed. The number of leukocytes in the cervical secretion is less than that of vaginal and uterine secretion, suggesting comparatively less phagocytosis at this site. The larger the reservoir, the longer it will provide spermatozoa for fertilization and hence the greater will be the chance of fertilization. Spermatozoa are released from the reservoirs and reach the site of fertilization through their own motility and uterine contraction.

The cervical mucus at the time of ovulation is composed of macromolecules arranged into micellar units. A physiologic balance of ovarian steroids is necessary for the initiation and maintenance of cervical population of spermatozoa following artificial insemination and estrous synchronization. An imbalance in progesterone and estrogen concentrations will alter the qualitative and quantitative characteristics of the cervical environment and spermatozoa transportation and storage. Uterine environment expose the spermatozoa to phagocytosis and the contractile activity facilitate transportation.

Oviductal transport of gametes occurs in opposite directions. This transportation is facilitated by paristalsis and antiperistalsis, contraction of the mucosal folds and the fluid currents and countercurrents created by cilliary actions. Ovarian hormones affect the structure, ultra-structure and secretary activity of the cervical, uterine and oviductal epithelia. Contractions of various components and secretions are also controlled endocrinologically.

SPERM CAPACITATION AND ACROSOME REACTION

Spermatozoa are synthesized by the testis and they must undergo maturation while passing forward through the epididymis. During ejaculation the spermatozoa get mixed with secretions from the accessory glands including prostate and seminal vesicles which support the initiation of motility; however, the spermatozoa still do not possess the capacity to fertilize an oocyte. After deposition of semen into the female reproductive tract during artificial insemination or natural services, the sperm move upward through the reproductive tract to the site of fertilization in the oviduct. During transportation, sperm cells undergo a series of biological reactions involving ionic, metabolic, and membrane changes that constitute the process of capacitation. Capacitation depends upon temperature and time that enables spermatozoa to get attached to the zona pellucida of the ovum, followed by the acrosome reaction.

The acrosome reaction is an exocytotic event by which proteolytic enzymes (acrosin and hyaluronidase) are released which enables a spermatozoon to penetrate the zona pellucida followed by fusion with the oocyte. Sperm capacitation takes place in the female reproductive tract and it can also be reproduced in vitro; however, this is a very complex mechanism and not yet fully understood.

The capacitation events comprise various biological changes like calcium influx, an increase in pH, rising levels of intracellular cAMP and production of low but regulated concentrations of superoxide anion ($O_2^{\bullet -}$). During capacitation the proteins from the fibrous sheath (located at the center of flagellum surrounding the anoxeme) undergo tyrosine phosphorylation (P-Tyr). This phenomenon supports the function of spermatozoa because the sperm cell has no capacity to synthesize proteins. Recent studies have revealed other phosphorylation events which have been associated with capacitation linking the earlier and later events of capacitation.

Reactive oxygen species (ROS) play and important role in the series of biological reactions leading to capacitation,the acquisition of fertilizing capacity. ROS have been described as injurious by-products produced as a result of cellular metabolism. However, when they are produced in lower concentrations, these ROS stimulate cell signaling events which in turn regulate physiological functions. Early studies have concluded that ROS, and especially H_2O_2, adversely affect semen motility and viability if produced at high concentrations in human and bull semen. However, recent studies have suggested that very low quantities of ROS support the process of capacitation and acrosome reaction, which are required for enabling spermatozoa to fertilize an ovum. $O_2^{\bullet -}$ supports capacitation and that it is released extracellularly by plasma membrane. Superoxide dismutase (SOD) has been found in high concentrations in seminal plasma of all mammals, and it seems to protect spermatozoa against oxidative

stress and premature capacitation. Capacitation may be induced in human and hamster semen through addition of ROS to the incubation medium.

Oviductal fluid (ODF) was found to support capacitation of spermatozoa in cattle, sheep and dogs. The ODF of various species of domestic animals contains a various types of glycosaminoglycans (GAGs). GAGs seem to promote sperm survival and capacitation and in vitro embryo production. The sulphated GAGs have a linkage with the proteins proteoglycans complexes.

Capacitation in the female genital tract takes place, mainly in the oviduct which enables the spermatozoon to fertilize an ovum. Capacitation comprises a series of biochemical reactions like destabilization of sperm membrane enabling spermatozoa to release their enzymes, which enables the spermatozoa to penetrate the zona pellucida. However, it is not yet clear exactly how, where and when capacitation takes place in the female reproductive tract. The knowledge generated so far in this area, is based upon in vitro studies with oviductal explants, which has led to the conclusion that sperm capacitation takes place in the sperm reservoir.

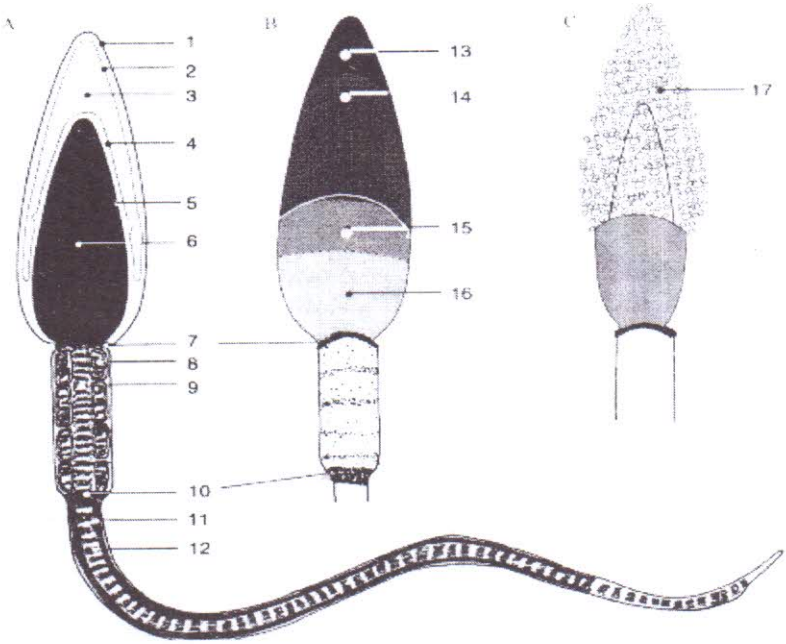


Fig. 3.13 A spermatozoon of horse. A: sectional view. B: Head of the sperm. C: the acrosome reaction. (1) plasma membrane; (2) outer-acrosomal membrane; (3) acrosome fluid; (4) inner-acrosomal membrane; (5) nuclear envelope; (6) nucleus; (7) nuclear ring; (8) mitochondria; (9) proximal part of the flagellum (mid-piece); (10) annular ring; (11) distal part of the flagellum (principal and end pieces); (12) fibrous sheath; (13) apical subdomain; (14) pre-equatorial subdomain; (15) equatorial subdomain; (16) post-equatorial subdomain; (17) mixed vesicles formed during the acrosome reaction via fusions of the plasma membrane with the outer acrosomal membrane. (Gadella et al., 2001).

In vivo, the oviductal fluid provides the environment for capacitation of the spermatozoa. Capacitation has been found to be irreversible process under physiological conditions; and that a capacitated spermatozoon must be located closer to the ovum so that it may interact with it.

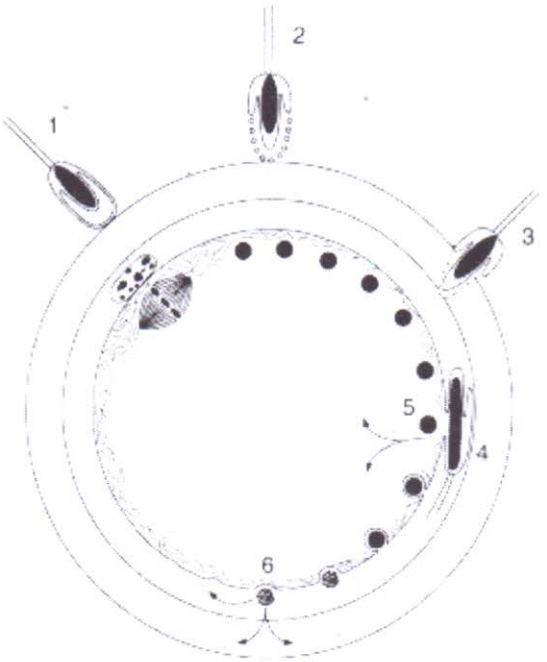


Fig. 3.14. Sequence of interactions between the male and female gamete required for fertilization: (1) sperm binding to the ZP with its apical subdomain; (2) the acrosome reaction; (3) the penetration of the sperm cell through the ZP (4) sperm binding and fusion with the oolemma (fertilization) (5) activation of the oocyte by cytosolic factors and fast poly-spermy block; (6) cortical reaction (Gadella et al., 2001).

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CHAPTER- 4

LABORATORY AND CLINICAL PROCEDURES

TABLE PALPATION OF THE FEMALE REPRODUCTIVE TRACT

Objectives

- A student is expected to recognize various parts of the reproductive organs.
- Normal reproductive organs and any pathological change should be noted.
- Recognizing textures and biometry to facilitate rectal diagnosis of various organs and their parts.
- Practical skills are developed for:
  - rectal palpation of living animals
  - insertion of AI rod for liquid semen
  - insertion of AI gun (insemination syringe for frozen semen)
  - rectal diagnosis of pregnancy
  - monitoring of postpartum reproductive states
  - administration of medicines and handling of obstetrical cases
  - understanding ultrasonography through echogenic areas
  - aspiration of antral follicles for procurement of oocytes

Procedures

- Proper protective clothes, gumboots and gloves are used to prevent getting any disease from the slaughtered animals.
- Reproductive organs are procured from slaughter house, preferably from different species, age groups and reproductive states, including adult cows.
- Reproductive organs are stored under refrigeration, examined in steel trays and dissected.
- Ovaries, infundibulum, oviducts, uterine horns, body and cervix are examined and information is recorded.

Ovary

- It is the primary reproductive organ situated at the end of uterine horns, supported by a fold of peritoneum called mesovarium.
- Its exocrine function is production of ova; and endocrine functions are production of steroid hormones for controlling activities of other organs.
- Germinal Epithelium is the outer superficial epithelium.
- Tunica albuginea is situated beneath the germinal epithelium.
- Cortex contains ovarian follicles.
- Medulla is composed of fibro-elastic connective tissues, nervous and vascular supply.

Structures to Be Examined

- Corpus hemorrhagic is the site of recent ovulation.
- Corpus luteum is a dark red, soft structure, producing progesterone.
- Corpus albican is the yellow colored, hardened, regressed corpus luteum.
- Antral follicles are fluid-filled vesicles which may be aspirated for harvesting oocytes to be examined or cultured and developed further.
- Primordial follicles and primary follicles are microscopic structures, containing oocytes.

Changes during the Estrous Cycle

- ***Proestrus:*** Developing follicles on ovary along with some antral follicles and a regressing corpus luteum may be seen.
- ***Estrus:*** One developed and functioning Graafian follicle may be seen on the ovary along with several antral follicles
- ***Metestrus:*** Corpus hemorrhagicum may be seen on the recently ovulated site or developing corpus luteum on ovary
- ***Diestrus:*** A functioning corpus luteum along with several antral follicles may be seen on surface of the ovary.
- ***Anestrus:*** No follicle or corpus luteum is present on ovary and the size is smaller.

Oviducts

- Extend from tip of the uterine horn up to the infundibulum, embedded in the mesovarium.
- Transport sperm to site of fertilization; prevent polyspermy.

- Remove oocyte from surface of ovary.
- Transport oocyte to site of fertilization.
- Provide environment for oocyte, sperm and fertilization.
- Transport & facilitate development of early embryo.

Structures to be examined

- Separate the oviducts from the mesovarium straighten measure and recognize various parts.

Uterus

- The uterine body measures 2-4 cm and uterine horns measure 35-40 cm in cow; lined with endometrium and smaller projections called caruncles, project inwards.
- Spermatozoa are transported up to the site of fertilization.
- Uterus provides proper environments for implantation and development of embryo and fetus.
- Uterus and cervix facilitate birth of fetus.
- Uterus helps in resuming postpartum reproductive cyclicity followed by conception, gestation and parturition.

Structures to be examined

- Palpate the broad ligament and its components, the mesovarium, mesosalpinx and mesometrium; which are an extension of the peritoneum folds attaching the ovaries, oviducts and uterus with the body wall.
- Palpate the caruncles and count them.
- Examine uterus for pus, pregnancy or any outgrowth, etc.
- Early pregnancy is found in reproductive tract.
- It is usually found in slaughter house organs.
- Placenta and placental fluids may be examined.
- Fetal cotyledons and maternal caruncles may be palpated.

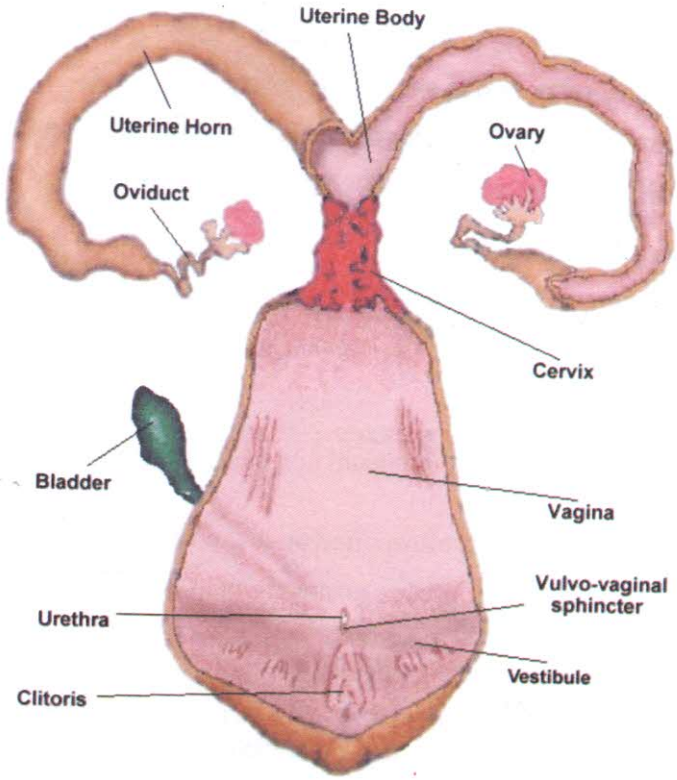

Fig. 4.1 Reproductive tract of cow (Geisert, 1999)

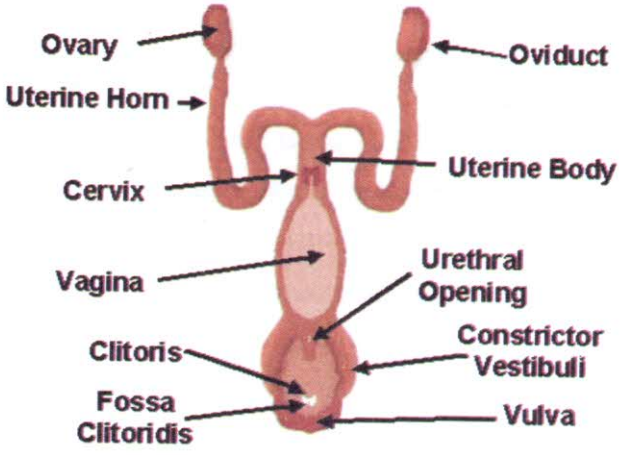

Fig. 4.2 Reproductive tract of bitch (Geisert, 1999)

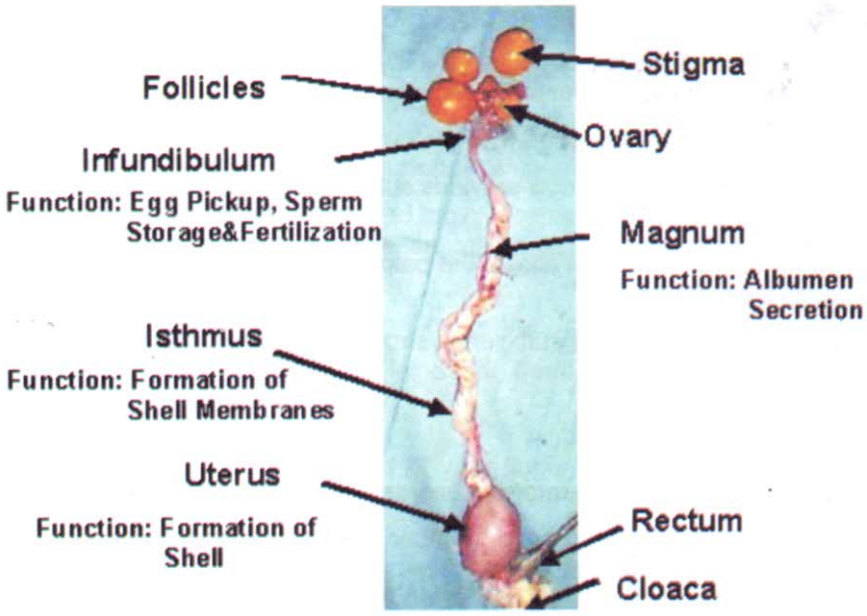

Fig. 4.3 Reproductive tract of hen (Geisert, 1999)

RECTAL PALPATION AND PREGNANCY DIAGNOSIS

Objectives

- Rectal examination is the only practical method for examination of the genital organs of cows and heifers of breeding age.

  It is used for:

  - Monitoring ovarian structures and stage of estrous cycle
  - Estrus detection and artificial insemination.
  - Diagnosis of pregnancy
  - Diagnosis of diseases of the genital tract
  - Ultrasonography for ovarian monitoring or early pregnancy diagnosis; followed by non-surgical recovery and transfer of embryos
  - Monitoring postpartum reproductive activities with a view to assess suitability of the animal for breeding and diagnosis of ovarian cyclicity.
  - Detection of cystic ovarian disease and infections of uterus; assisted parturition, treatment of dystocia and the torsion of uterus

- Rectal diagnosis of pregnancy provides immediate results, no extra inputs are needed the accuracy goes up to 95%.
- Early treatment of non-pregnant animal is ensured.

Anatomical Basis

- The broad ligament suspends the reproductive tract of cow from the dorsal body wall.
- Posterior part of the reproductive tract is located inside the pelvic cavity while anterior part is hanging over the pelvic brim.
- The reproductive tract is located just below rectum.
- The size of the rectum of cow provides sufficient space to the palpator for hand insertion up to arm.
- Pliability of the rectal wall allows the palpation to grasp the reproductive tract through the rectal wall.
- In cows the rectal wall is sufficiently strong which prevents its tearing during palpation. In species like mare, the rectal wall is weaker and may be damaged during palpation.

Procedures

- Breeding history of the cow may be recorded.
- Record date of the last calving, number of services, and information on any pathological condition or disease.
- Examine the tail head and dorsal lumbar area to determine if the hair is roughed-up or lost as a result of the cow being mounted.
- Commonly rectal palpation is made for pregnancy diagnosis beyond 60 days; while experience practitioners may do it beyond 35 days post-breeding.
- Examine the perineum and thighs lateral to the vulva, as well as the under side of the tail for mucus or blood.
- Examine the vulva tuft of hair for the presence of small amounts of menstrual bleeding. This is present 1 to 3 days post-estrus in 90% of first service heifers and only 50% of the time in multiparous animals.
- Part the lips of the vulva and examine the mucous membrane. When estrogen levels are low, the membrane is pale and wet, but not shiny.
- Large amount of glistening mucus that adheres to the walls of the vulva during heat, and imparts a pink color to its wall.

Restraint of Cow

- Essential to avoid injury to the examiner, the attendants and the animal.
- A cattle crush (chute or stanchion) is used.
- Fright to the cow should be minimized through proper approach; sudden movements may be avoided and acting in a loud, frightening way may be avoided when the cow is not exposed to people.

- Kicking by the dairy cows is not common, how ever some cows will kick. If a cow is approached slightly from the side such kicking may be avoided.
- Touch the cow gently on the flank or back so that it gets awarded of presence of the palpator and temperament of the cow are evaluated.

Requirements

- Wear protective clothes before palpating an animal per rectum.
- Wear a well-fitted sleeve and glove; "one size fits all"; because of the risk of exposure to zoonotic disease, cattle should not be palpated without a protective sleeve and glove.
- After donning the palpation sleeve, the glove, a non-irritating water-soluble lubricant, such as lubrivet. K-Y jelly or a commercially-available lubricant may be used. It will facilitate easier entry into the rectum and will further reduce the danger of injury.
- Measure the relevant parts of your finger to assess various dimensions of ovaries, etc. during rectal palpation (2.5 cm in my case).

Insert Hand into the Rectum

- Form the fingers into a cone and gently but firmly insert it into the anus.
- The presence of the hand in the rectum stimulates the defecation reflex which manifests itself in peristalsis as well as tenesmus.
- Discontinue examination during a peristaltic wave and a wave must never be allowed to pass over the hand. Instead, the wrist should be brought back to the anus but it should not be removed.
- Remove feces from the rectum for nearly the entire length of the arm without allowing air to enter.
- Aspiration of air into the rectum causes "ballooning" which absolutely prevents examination. Reach forward and gently grasp the first peristaltic ring of mucosa then slowly pull it backward.
- Removing feces for almost the entire arms length provides surplus rectum which will permit greater ease in performing manipulations of the genital tract.

Palpation Techniques

- Use the cervix as the landmark for locating the internal genital tract; locate cervix after emptying the rectum.
- Cervix is recognized as a firm, cylindrical, somewhat nodular structure and is usually found lying on the midline on the pelvic floor, at or just cranial to the brim of the pelvis.

- Examine the entire pelvic inlet to locate the cervix. Once located, the cervix should be examined for size, form, position, consistency and motility.
- Indirect method: First locate the cervix and ensure whether it is freely movable, then pull it back as much as possible. Try to bring up the uterus and closer through movement of the cervix upward and towards the wall of the pelvic cavity opposite to the palpating hand.
- The uterus is held in this position through placement of thumb below the body of the uterus.
- Then grasp anterior edge of the broad ligament, by keeping the thumb in the above-mentioned position; then turn the hand outward, the bent fingers are lowered and the broad ligament are hooked from underneath. After following this procedure, the broad ligament is held between the ovarian end of the uterus and the ovary.
- It is then lifted dorsally and drawn caudally. The bent fingers are then slid medially, and the uterine horn is gathered in the palm of the hand. The uterine horn is pulled caudally and the fingers are slid further medially until a firm grasp of the ventral inter- cornual ligament is obtained.
- Further retraction of the uterus is accomplished with gentle traction on the ventral intercornual ligament.
- Direct retraction: Locate the cervix and pull it as far caudally as possible. The dorsal intercornual ligament is then located and grasped. Slight caudal traction is exerted to bring up the ventral ligament which is grasped, and the retraction is completed as described for the indirect method.
- Once the uterus is retracted, the uterine horns should be palpated, their entire length for size, tone, and consistency. If the horns are empty, they will have a meaty-like consistency.

Pregnancy Diagnosis per Rectum in Cow

- Diagnosis of pregnancy at an earlier stage improves reproductive efficiency and it leads to an improved conception rate in cows by shortening the postpartum breeding interval.
- Rectal palpation is the most widely used diagnostic technique for pregnancy, and is faster and cheaper.
- It may be performed during 35th day onwards by skilled practitioners and 60th day onward by less skilled ones.
- Pregnancy diagnosis per rectum is based upon:
    - Asymmetry of the uterine horns, the pregnant horn manifesting more enlargement than the other one.
    - Differentiation of the enlarged horn from the urinary bladder, confirming its continuation with the cervix.

- Slip membrane test involving slipping off of the fetal membranes and the uterine wall moving through the fingers while grasping the wall of the rectum and letting them gradually slipping through the fingers. It is also called as the "fetal slip".
- The calf fetal size may be detected as mouse, rat, cat and dog during 2, 4 and 6 months post-breeding, respectively.

Early Pregnancy Diagnosis

- Pregnancy may be diagnosed per rectum as early as 35 days post-breeding. Negative pregnancy may be declared carefully.
- Pregnancy may be based on a combination of the following:
  - Asymmetry of the uterine horns due to accumulation of fetal fluids in the pregnant uterine horn.
  - Decrease in the tone of the pregnant horn; fluctuant contents in the pregnant horn (later both horns)
  - A palpable corpus luteum on the ovary on the same side as the pregnant horn.
  - Fetal membrane slip may be detected during 30-35 days, amniotic vesicle during 35-60 days and the fetus during 65+ days; in-experienced persons may avoid earlier pregnancy diagnosis per rectum.

Diagnosis of Advanced Pregnancy

- Pregnancy more than 3 months post-breeding may be diagnosed per rectum by less skilled workers.
- Cervix is located anterior to the pelvic brim and the uterus cannot be retracted.
- Uterus is flaccid; placentomes and/or foetus, are palpable.
- Uterus is pelvic up to 60-90 days and abdominal later on.
- The median uterine artery increases in diameter and fremitus can be detected.
- Fetal reflexes may be detected by touching its head and eyes and a living fetus may be confirmed.
- Deeper palpation will be required; in case of no access to the fetal body, the presence of cotyledons, fremitus and slip membrane test may be indicative of positive pregnancy.
- Pyometra may be diagnosed as false pregnant and may be differentiated through the presence of cotyledons, fremitus and slip membrane test.

Economic Implications

- On confirmation of negative pregnancy, the cows are culled.

- Fate of the bull about its use for breeding purpose is decided before commencement of the next breeding season.
- The cow is managing in different ways during different stages of pregnancy. For example, the cows expected to calve during later time in the season may be culled, sold to another farmer, or provided a different feeding plan.

Precautions and Safety Measures

- False negative pregnancy may be declared due to failure to retract the uterus.
- False positive pregnancy may be declared due to abnormal uterine contents like pyometra or mucometra.
- Incorrect service dates may lead to false results.
- Early or inappropriate palpation of the amniotic vesicle may damage the embryo and cause embryonic mortality.

Table 4.1 Accuracy of various techniques for diagnosis of pregnancy

Technique	Early testing	Positive diagnosis accuracy	Negative diagnosis accuracy
Palpation per rectum	+	+++	++++
Ultrasonography per rectum	++	++++	++++
Milk progesterone profiles	+++	++	+++
Early Conception Factor	++++	+	+

Table 4.2 Positive signs of pregnancy at rectal palpation

Day post-breeding	Membrane slip	Amniotic vesicle	Foetus	Place-ntomes	Fremitus
30	±	+			
45	+	+			
60	+	+			
75	+	+		+	
90	+		+	+	
105			+	+	+
120			+	+	+
150			+	+	+
180				+	+
210			+	+	+

Pregnancy Diagnosis Per Rectum In Mare

- The cervix becomes elongated, firm and tubular from 16 or 17 d to term.
- The uterus has increased tone from 16 or 17 d to term.
- The chorionic vesicle may be palpated like a spherical body measuring about: 28 days: egg of a pullet; 35 days: size of a lemon; 42 days an orange size; 49 days a grapefruit and; 56 days the size of a small ribbed melon.
- Up to 90 days the fetus is hard to delineate the anterior portion of uterus.
- Ballottement of fetus per rectum is possible after 150 days.
- False positive results may be given due to palpation of bladder and swelling of the non pregnant horn of the uterus.

Laboratory Diagnosis Of Pregnancy

- Milk progesterone is detected through enzyme immunoassay (EIA) or radio immunoassay (RIA) for pregnancy diagnosis on 21 days post-breeding; the negative results are highly accurate while the positive results may be confused with luteal phase.
- EIA may be used in any laboratory and the competitive binding between the sample progesterone and the label progesterone results in development of color which is inversely proportional to the quantity of progesterone in the sample.
- RIA is more sensitive and it uses gamma counter for detection of the progesterone in the sample and the technique needs special arrangements and authorization for handling radioactive materials.
- Oestrone sulphate is a metabolite of the oestrogens produced by the foeto-placental unit. From 7 weeks of pregnancy onward, oestrone sulphate levels in maternal blood are sufficient to serve as an indicator of pregnancy.
- Since the oestrogens in goats are eliminated via the faeces, it is possible to measure faecal oestrogen levels in three or four dung pellets collected from the rectum.
- Pregnancy associated glycoprotein (PAG) in blood is produced by multinuclear placental cells, and its concentration in maternal blood increases with pregnancy.
- At 3–4 weeks of pregnancy plasma PAG levels differ sufficiently from that of non-pregnant does to serve as a basis for a pregnancy diagnosis.
- Although concentrations of PAG in milk are 10 times lower than in blood, milk samples may be useful for diagnosing pregnancy from day 32 after breeding onward.
- Transducer provides a reliable pregnancy response as early as 20 days after service in goats detecting the presence of a fluid-filled vesicle in the uterus, and from day 23 onward when insisting on the identification of fetal heart beat.

Palpation of The Ovaries

- Bovine ovaries without structures are oval in shape and weigh 5 to 15 grams each.
- Older cows usually have larger ovaries than younger cows.
- The ovaries are usually located in the ovarian bursa, a pocket formed by the proper ligament of the ovary and the mesovarium, at the anterior edge of the broad ligament.
- The ovaries can usually be located slightly cranial to the pelvic inlet and slightly cranial and lateral to the internal opening of the cervix.
- They are easily located by grasping the anterior edge of the uterus.
- Left handed palpators can locate right ovaries more readily and right handed palpators can usually more readily locate left ovaries.
- The ovaries are pulled ventrally as the uterus enlarges.
- The experienced palpator should identify and differentiate the following structures on the ovaries of most cows:
    - Ovulation depression (OVD)
    - Corpus hemorrhagicum (CH)
    - Corpus luteum (CL)
    - Follicle (F), and
    - Ovarian cyst (C).
- Before making a diagnosis, the palpator should be sure that the structures palpated are on the ovary.
- The stage of the estrous cycle and the approximate days until the next expected estrus can be estimated fairly accurately by the palpator.
- Ovarian findings, tubular genital tract findings, physical condition of the cow and reproductive history should be considered prior to predicting the time of the next estrus.

Estrus Detection

- Estrus is characterized by an decreasing progesterone and increasing estrogen produced by the developing follicle.
- Inadequate heat detection is a greater reproductive problem and missed heats lead to delayed postpartum conception.
- Calving interval may be reduced by increasing the number of observed estruses.

- With an increase in production, conception rate and heat detection efficiency increase indicating that greater milk yield may be coupled with good reproductive performance.
- An efficient procedure of heat detection assists a farmer to get superior genetics available through AI.

Estrus Symptoms

- Cows and buffaloes should cycle every 21 days.
- A cows should exhibit estrus by 50 days postpartum and buffaloes by 70 days.
- Standing to be mounted by a herd mate has been found as the most reliable sign an estrous cow. Each standing remains for a period of only 4 to 6 seconds. The number of mounts in a cows have been found to be about 1½ mounts per hour and the cows exhibit estrus after 6-8 hours.
- Buffaloes show about 50% silent ovulations, which may only be detected through intact bull or hormonal assays.
- Crossbred cows show prolonged estrus sometimes, due to delayed ovulation or anovulatory estrus.
- Heat detection must be done daily, accurately and recorded properly.
- Cows must be observed for heat several times daily.
- Secondary signs of heat may be monitored, including:
    - mounting over other cows in the herd
    - discharge of a clear vaginal mucous
    - resting of chin and rubbing
    - vulva swollen and red in color
    - frequent micturition
    - head of tail ruffled and flanks muddy
    - sniffing, bawling and restless behavior
    - lower milk yield and appetite

Estrus Detection Aids

- **Estrotect Heat Detectors** (Estrotect, Inc) is a cheaper, user friendly and highly effective aid for detection of heat.
- It provides a simple technology of "scratch-off" associated with better results for estrous detection.
- The kit is provided in four fluorescent colors – green, blue, fuchsia and orange.

- Estrotect may be utilized as a technique in detecting estrous animals for artificial insemination or monitoring recipients in embryo transfer programs.
- Apply once per cycle. Alternative tail paints and paint sticks must be applied or touched up daily.
- *Detect advancing heat. Other detectors are activated by one mount, sometimes for as little as 3 seconds.*
- Estrotect can minimize the number of false positive cases due to product design that scratches off and works over time.
- Increased accuracy. Heat detection accuracy using scratch-off technology is greater than 90% when compared to vigorous visual observation and penile deviated bulls.
- **Progesterone test** is based upon detection of the hormone through milk or blood enzyme immunoassay or radio immunoassay.
- After a period of high progesterone produced by the corpus luteum, a low progesterone level suggests a cow or buffalo has finished her cycle and should ovulate and show heat.

OvSynch

- **For dairy cows** Synchronized Ovulation Protocol (OvSynch) was developed and field tested during 1989, 1997 and 1999 (Figures 4.4 and 4.5).
- It comprised controlling the estrous cycle through exogeneous hormones.
- A GnRH injection is given followed by luteolysis through PGF2α on day 7.
- On next day GnRH is repeated to induce LH surge.
- Then the cows are inseminated at a fixed time during 0 to 32 hours after second injection of GnRH, while highest conception rates can be achieved 16 hours after injection of GnRH.
- Real conception rates have been found to be lower after fixed-time artificial inseminations, but the rates are greater when the animals are inseminated following detected estrus.
- Behavioral estrus detection have led to lower pregnancy rates in programmed breeding systems while fixed time insemination resulted in greater rates of conception.
- **For dairy buffaloes**, Ovusynch-Plus' Protocol was tested in buffaloes at Central Institute for Research on Bufffaloes, Hisan India.
- It gave encouraging results in management of anestrus and subestrus with good fertility to fixed time inseminations, thereby circumventing the need for estrus detection, which is otherwise inefficient in buffaloes.

- **Comparison with standing heat methods:** Ovsynch has been used in repeat breeders having no heat signs or cystic ovaries. Concpetion rate after use of Ovsynch was recorded as 38%. Pregnancy rate on "normal cows" after synchronization was 33%, when compared with the standing heat pregnancy rate of 67%. Use of ovusynch in repeat breeding cows was almost equal in efficiency to the normal cows.

- Cows are selected for using Ovsynch method when need arises. However, efficient heat detection regime can never be replaced by any other technique in dairy cows.

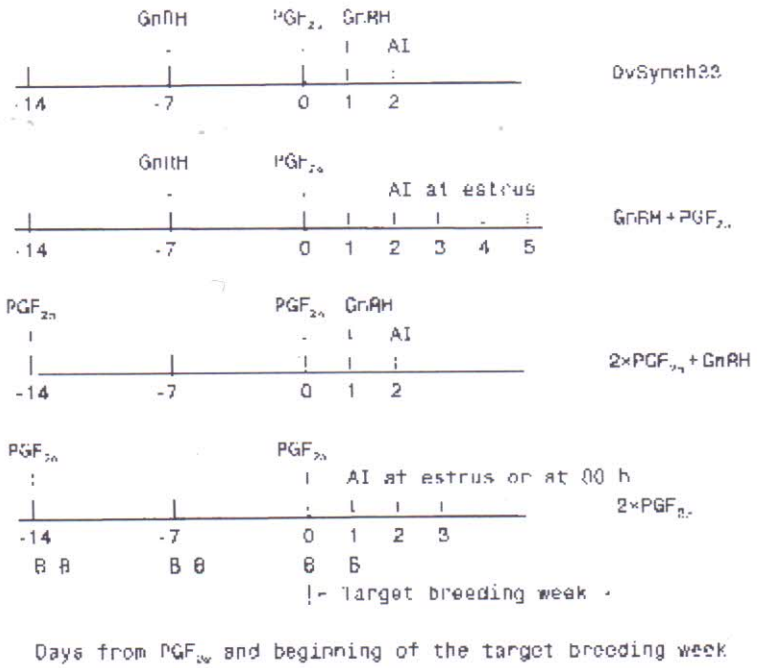

Figure 4.4 Synchronized ovulation protocol 1. (OvSynch 33: GnRH injection at 33 h after PGF2a)

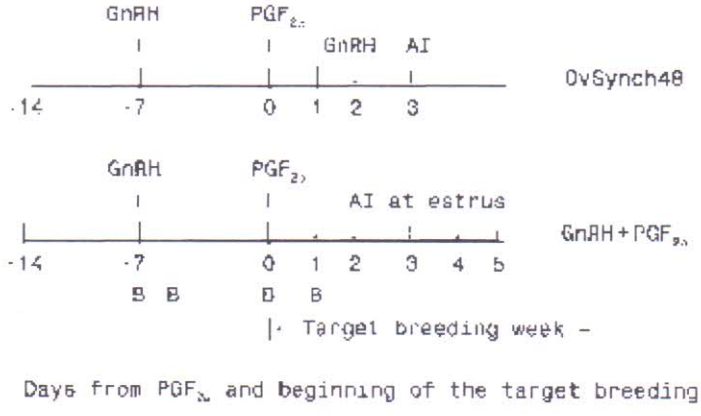

Figure 4.5 Synchronized ovulation protocol 2. (OvSynch 48: GnRH injection at 48 h after PGF2a) Source: Stevenson et al. (1999)

THE LACTATING AND NON-LACTATING UDDER

The Lactating Cow's Udder

- *The udder comprises four quarters containing a ducts of various sizes in form of a complex network of blood vessels, capillaries, ligaments and lactogenic tissue (Fig. 4.4).*
- As the udder produce and store the milk between milking, it should be stronger and properly attached to the belly.
- Age and inheritance of the cow will affect the size and shape of udder, up to a considerable extent.
- The udder comprises two halves separated by a supporting ligament which each half is divided into two quarters by a membrane.
- Although every quarter is separate entity allowing no transfer of milk between quarters, however, antibiotics can transmit from one quarter to another.
- When a cow gets treated with antibiotics in one quarter, the milk from all quarters may be discarded.
- Administration of antibiotic into the muscle, etc., will also transmit into the milk through blood circulation.

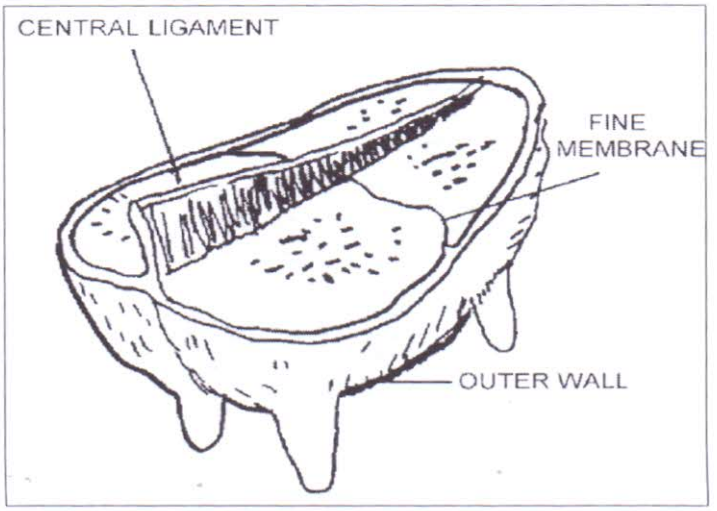

Fig. 4.6 The cow's udder (Bray and Shearer, 2003)

The Alveolus

- The alveolus constitutes the basic unit for milk secretion.
- It comprises epithelial cells which surround the lumen.
- Various milk components comprising protein, fat and lactose, are synthesized from metabolic precursors in the blood circulation and secreted into alveolar lumen.
- Muscle cells surround the alveolus and these are called as myoepithelial cells.
- The myoepithelial cells are stimulated by oxytocin followed by milk ejection from the alveolar lumen.
- After contraction of the alveolus milk is comes out through capillary milk duct which evacuate into a series of milk ducts getting progressively larger until the milk is collected into the gland and teat cisterns.
- Myoepithelial cells also surround the smaller ducts of mammary gland and help in milk ejection.

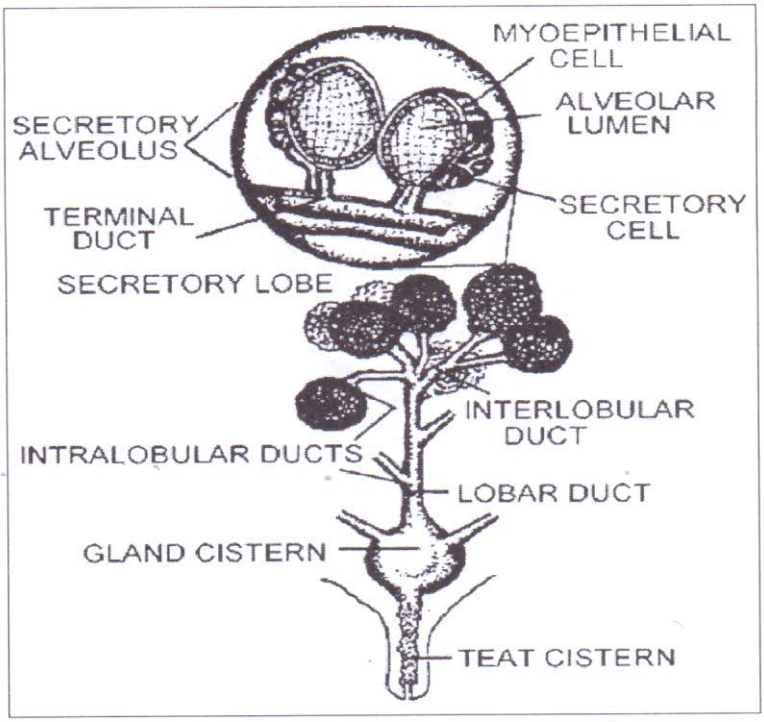

Fig. 4.7 Secretary lobes and ducts of udder (Bray and Shearer, 2003)

Teat Histology and Mastitis

- Elastic connective tissues are found around the teat wall in addition to the two layers of muscular tissue.
- The teat canal is lined with epithelial cells in addition to a thick layer of keratin, the flat cornified epithelial cells. The layer prevents the transmission of infectious organisms to udder.
- After onset of mastitis infection the lymph nodes get stimulated, increase the release of lymphocytes into the blood which are transferred to the udder to prevent mastitis.

Milking and the Process of Milk Letdown

- After proper stimulation, the cow lets down the milk.
- Stimulation may be achieved through washing or massaging the udder, removal of the fore-stripping from each quarter or suckling by a calf.
- Stimulation of udder sends nerve impulses which cause the pituitary gland to release oxytocin into the blood circulation.
- Oxytocin causes contraction of myo-epithelial cells around milk-filled alveoli which forces the milk into milk ducts and teat cisterns.

- In case of any pain or excitement of the cow adrenaline is released to the blood stream which reverses the letdown of milk.

The non-Lactating Udder

- The cow stops lactation about 40 to 60 days before parturition, a condition known as dry cow.
- A cow with hard and swollen udder should be milked for removal the microorganisms and toxins which may lead to mastitis.
- Long-acting antibiotics are infused into each quarter of the udder at the end of lactation, known as dry cow therapy.
- In case of sub-clinical mastitis only cows with a record of mastitis or those having high somatic cell counts, are treated.
- As there is no milk production during the dry period, antibiotics may be used in higher dosages without any risk of drug residues because the will remain in the non-lactating udder for longer time. Other therapeutic measures like vaccinations and de-worming may be used safely during the dry period of a cow.

THE MALE REPRODUCTIVE TRACT

Testis and Scrotum

- Each testis is contained in scrotum suspending as an out-pouch of the peritoneal cavity and comprising of a fold of the skin.
- Like rest of peritoneum, the scrotum is lined with serosa which consists of mesothelial cells. These cells are supplemented by fibrous connective tissues as shown in Fig. 4.8.

Tunics

- The testis is surrounded by a layer of peritoneum called as tunica vaginalis.
- Tunica albuginea is the name given to the visceral peritoneum is named tunica albuginea.
- Both of the two tunics are composed of fibrous connective tissue covered with a thin mesothelium.

Mediastenum

- This is the thick portion of tunica albuginea located posteriorly.
- Mediastinum receives the nerves, arteries, veins, lymphatic vessels, and semen collecting ducts for the testis.
- Fibrous septa initiate at the mediastinum and enter into body of the testis.

Seminiferous Tubules

- Seminiferous tubules constitute the bulk of each testis and these tubules are embedded in interstitial tissue (Fig. 4.9).
- The tubules produce spermatozoa and hormones are synthesized by Leydig cells located in the interstitium.
- The tubules are different from those in a typical exocrine gland, because each seminiferous tubule makes a tightly coiled loop, measuring about a meter and opening into the rete testis at both ends.
- Atrophy of the testicular tubules may lead to infertility associated with cryptorchidism or orchitis (inflammation of testes).

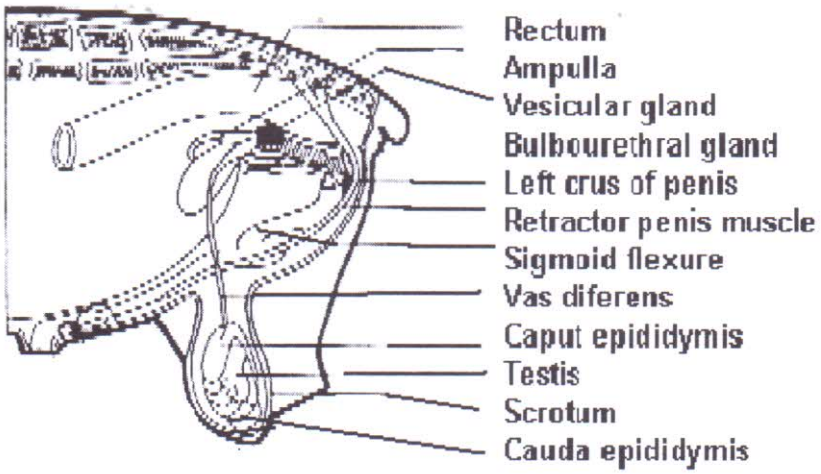

Figure 4.8 The reproductive system of the ram (Redrawn from Sorenson. 1979)

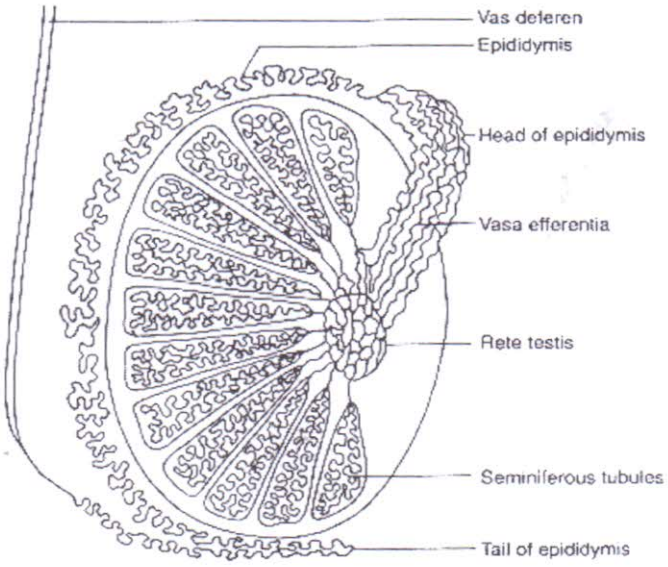

Figure 4.9 Sagittal section of testis.

Spermatic Cord

- The spermatic cord provides continuation of the testis to the convoluted testicular arteries which are surrounded by venus plexus, and the nerve trunks.
- It is constituted by connective tissues, smooth muscle fibers, and a part of the vas deferens.
- The spermatic cords and the scrotum provide support the testes and help in regulation of temperature.

Epididymis

- Epididymis is an external duct leaving the testis and is fused to the surface of the testis in a longitudinal pattern. It is enclosed in the tunica vaginalis.
- Caput epididymis is located at the apex of testis, and is a flattened body which recieves 12 to 15 small ducts known as vasa efferentia and they merge into a single duct.
- Corpus epididymis is the body of epididymis and it continues along the longitudinal axis of the testis ending up at the cauda epididymis.

Accessory Glands

- Accessory glands are found in the pelvic portion of urethra, and they provide ducts which release their secretions into urethra.

- Vesicular glands, the bulbo-urethral glands and prostate gland are accessory glands and they contribute to volume of the semen.
- These secretions provide nutrients, buffers and other components supporting optimum motility of spermatozoa and fertility.
- Vesicular glands also known as seminal vesicles are shaped like cluster of grapes and contain fructose and sorbitol. These two substances provide energy for spermatozoa in bulls and rams.
- The prostate gland is found as a single body and is surrounds the urethra, located posterior to the excretory ducts of the seminal vesicles. Secretions of this gland are high in inorganic ions.

SEMEN COLLECTION

- Dairy cows all over the world and dairy buffaloes in the Asian countries, are artificially inseminated.
- Artificial insemination (AI) has also been introduced in other species and used for education and research purposes.
- AI is considered better than natural service because it ensures rapid genetic improvement using only the best selected bulls.
- Sometimes the AI bulls sire more than 100,000 progeny through artificial insemination.
- Electro-ejaculation may be used instead of artificial vagina when the bull cannot mount for semen collection or it is too fractious for easy handling.
- As a final attempt, semen can be collected through rectak massage of seminal vesicles and ampullae.

Use of Artificial Vagina

- Artificial vagina (AV) is used to get semen from trained bulls.
- Use of AV requires three persons for handling the teaser animal, controlling the bull and semen collection.
- The collection area must be having a non-slip flooring so that injuries could be avoided.
- Due to heavy weight the bulls must be provided regular hoof care. Poor hoof condition may prevent the bull from mounting and dismounting due to painful conditions.
- The AV may be properly washed through detergent and running tape water, rinsed with distilled water, sterilized under steam, dried in an oven under low temperature and stored under sterile conditions until use.
- Apply AV cover and collection tube cover to protect the semen and the AV from variation in temperature.

- The technician and the sire handler may be sufficiently trained to do their job properly avoiding any injury to themselves, the animal and the equipment.
- A separate and sterile AV should be used for collecting every ejaculate of semen.
- Bull may be collected twice a week getting two ejaculates during each collection. This will provide sufficient spermatozoa concentration and good semen quality.
- Keep temperature according to individual need of the sire. Usually a temperature of 42 to 450 C is used successfully in bull and buck.
- Keep the AV parallel to the path of the penis and guide the penis into the AV with the help of your free hand.
- Touch the AV with the tip of the penis and allow the sire to insert its penis into it through a forward jerky movement.
- Then tilt the AV downward so that the semen can run into the collection tube.
- Adjust the conditions of AV and the semen collection procedure with the individual sexual behavior of the sire.

The Teaser

- Steer is used commonly as a teaser for mounting of the donor bull.
- Use of females as teaser animal is avoided due to the potential risk of intromission of the penis, spread of venereal disease and weak stature of the females.
- The teaser animal should possess calm behavior with appropriate size for the donor bull.
- Back and rear quarters of teaser may be washed through an appropriate disinfectant solution on the day of collection. The rear quarters should be clipped as a routine practice.

Semen Collection

- Semen is collected from the bull twice a week with 2-3 ejaculates per collection.
- Preputial hair may be removed while preparing the bull for using artificial vagina so that microbial contamination of semen may be minimized.
- During semen collection, the teaser animal is positioned in the front of the donor bull to be mounting.
- Libido of the donor bull is stimulated if the steer is led around in the semen collection arena while the donor bull moves behind and then the teaser is stopped abruptly. It is similar to the behavior of an estrous cow.

- Principal of the artificial vaginal is the thermal and mechanical stimulation to the penis of the bull for stimulation of semen ejaculation.
- Inner liner of the artificial insemination is filled with warm water at 42-45 °C.
- Inner surface of the liner is lubricated with a lubricant like K-Y jelly. The author has noted that the jelly reduces semen quality so use of jelly is not recommended.
- The artificial vagina is covered in an insulating cone at the end of the semen collection tube to prevent semen from temperature shock.
- Sexual stimulation is aroused through false mounting of the bull. Two false mountings with a duration of restraint of two minutes followed by an additional false mount are recommended.
- Preparation of AV in a proper way is important to prevent damage to the penis of the bull and to avoid sperm cells from stress.

Safety precautions

- The AI bull should not be excited with the people, and it should have been trained for semen collection through AV.
- AI bulls are heavy and mostly vicious; so personnel safety should be given proper consideration.
- During semen collection, the technician handling AV should remain alert about position of their feet relative to the bull.
- During ejaculates, it is common for the bull to jump forward. Semen collectors must wear boots to avoid injuries.

Preparation of Extenders for Goat Semen

- Semen is diluted with extender to get maximum doses.
- A semen extender has the following characteristics:
    - Provides nutrients as a source of energy.
    - Prevents the harmful effects of rapid cooling.
    - Prevents effects of changes in pH.
    - Maintains osmotic pressure/electrolytes balance.
    - Inhibits bacterial growth.
    - Increases volume of the semen/semen doses.
    - Protects the sperm cells during freezing.

SEMEN EVALUATION

The Bse Criteria

- The American Society for Theriogenology has recommended the following criteria to standardize BSE (Breeding Soundness Examination) for veterinarians of the North America and to make available a set of standards which are acceptable under the law for bulls comparison (LSU, 2006).
- For qualifying the BSE criteria a bull must provide semen with normal physical examination and the scrotal circumference should meet the minimum requirements set for motility of spermatozoa and sperm morphology. These parameters may be tested and interpreted in an easy way.
- Required level of individual motility of the semen is > 30% to be passed. Motility of semen is the most lenient criteria, and can be influenced by the way the technical handles it. Proper procedure and laboratory environments are needed for getting proper results.
- Morphology of the spermatozoa must show >70% normality to get passed. Minimum criteria for the scrotal circumference must be met, which varies with the individual age groups.
- The candidate bull should meet all the minimum criteria set for that category, in order to get passed a BSE. They may b:
    - Satisfactory – when the bull has passed all the three criteria and it will be called as a good bull.
    - Deferred - the bull has failed to pass all criteria, and it will be re-evaluated, because it is expected that the problem may get corrected with the passage of time.
    - Unsatisfactory - the bull has failed to pass all criteria and the problems are not expected to be resolved.
- The examiner pulls all the laboratory and clinical information about the candidate bull and decides to pass or fail a bull for any valid reason.

Semen Evaluation Techniques

- Various techniques and assays have been developed and advocated for assessing semen quality, based upon three core parameters; the semen concentration, motility and morphology.

Routine Tests

- The semen collected at a semen production laboratory must have the capacity to fertilize a female of the same species and to genetically improve it.

- For this purpose various tests are conducted in the laboratory at various intervals.
- Some tests like examination of a drop of whole semen for concentration and mass motility, just after collection, is done after every collection; while other tests are conducted at various intervals according to the needs from the field.
- **Volume:** Volume is an important indicator of bull fertility and is recorded by examining the graduated semen collection tube.
- Volume can be increasing by increasing the false mounts.
- A very small volume of semen is discarded as it is not economical to be processed further.
- **Colour:** The normal colour of semen is like that of the whole milk. A yellow colour may be found in semen which is due to the lipochrome from the ampulla.
- Brownish colour indicate cold abscess; yellow colour shows pus flakes; yellow color on staining shows bacterial load; and dark red color is due to blood.
- A semen sample with abnormal color should be rejected.
- **Density:** Density or viscosity or consistency reflects the semen concentration varying from thick creamy to watery according to the following table:

Table 4.3 Density and concentration of semen from various species

Density	Sperm number (million/ml)	Grade
Thick creamy	> 2000	4
Creamy	1500-2000	3
Thin creamy	400-800	2
Milky	150-350	1
White	< 150	0

- Density of semen is observed in against normal light.
- Viscosity is recorded through running the semen against wall of the test tube and observing its grading as slow viscous (4, 3), slow running (2), rapid running (1) or completely running (0).
- **pH:** pH of semen shows the alkalinity or acidity and varies due to semen concentration and secretion from the accessory glands.
- Normal pH of bull semen is 6.7 and increase in sperm concentration may decrease the pH to 6.5.
- Storage of semen at room temperature may decrease the pH due to production of lactic acid from fructose.

- Alkaline pH is due to inflammation of the genital tract, azoospermia, dead spermatozoa and high bacterial load.
- pH is measured through pH paper or an electronic pH meter.
- **Mass motility:** It is the overall initial motility of the semen just after its collection and is determined through microscopic examination of an undiluted semen sample.
- Mass motility may be assessed through recording the intensity of waves of semen graded between 4 (80%) and 0 (0%).
- The minimum critical value is determined by each laboratory depending upon the quality standards.
- Semen ejaculates below the minimum values are discarded.
- **Individual motility:** It is the percentage motility of individual semen cells in the sample.
- The semen is diluted at a rate of 1:5 to 1:20, depending upon the concentration and a drop of the diluted semen is placed on the glass slide, covered with cover slip placed on a warm plat (or warmed on flame) and examined under the microscope at a magnification of 1 x 40.
- The number of progressively motile sperm is counted in a given field against the total spermatozoa.
- **Concentration of spermatozoa:** The concentration of spermatozoa is visually assessed by the intensity of darkness of a drop of the undiluted semen under the microscope.
- It is based upon experience of the evaluator and varies from the maximum value for the species (1500 million for buck or 1200 million per ml for bull) to the minimum value, i.e. 0.
- Determination of semen concentration helps us in dilution of semen for further use.
- Concentration is measured through visual observation as described above, or haemocytometer or spectrophotometer.

Periodical Tests

- **Sperm count on haemocytometer:** After collection, the whole semen is diluted 20 folds (dilution factor, 20) with spermicidal solution and processed as follows:
    - One drop of semen is added to 19 drops of spermicidal solution or 0.1 ml of semen is added to 1.9 ml of spermicidal solution. (Spermicidal solution contains triphenyltetrazolium chloride in physiological saline, 5% chlorazine in physiological saline, 5% sodium bicarbonate and 1 ml of

35% concentrated formaldehyde made up to 100 ml in physiological solution.).

- The semen is thoroughly mixed with the spermicidal solution and one drop is added to both sides of a standard blood cells haemocytometer.
- Spermatozoa are allowed to settle in a wet chamber.
- The spermatozoa in the five major squares A through E are counted under light microscopy using 100 or 400 magnification. A through D are the outer squares and E is the central square. Spermatozoa lying inside the squares and those touching the upper and right lines are counted. Both sides of the haemocytometer are counted and the average is calculated.
- The thickness of the fluid between the cover slip and the haemocytometer is 0.1 mm and the multiplication factor is 10,000 if counting is made in the major squares and 50,000 if the counting is made in minor squares. It is multiplied by the dilution factor which gives us the concentration of spermatozoa per ml in the semen.

- **Motility index (MI):** It is a precise measurement of motility of spermatozoa as the visual method may lead to errors.
- The motility index has got a higher correlation with live spermatozoa and cold shock resistance test.
- The semen is diluted in egg yolk citrate extender so that the concentration of the diluted semen is 10 spermatozoa per ml.
- The haemocytometer is filled with the diluted semen and examined under microscope. The number of spermatozoa crossing the 0.25 mm WBC line in any WBC chamber is accounted for. The counting is repeated 3 times and average is calculated. The count is multiplied by 107 and divided by the total number of spermatozoa/ml of diluted semen to get MI.
- **Live-dead count:** Semen contains live and dead spermatozoa in each ejaculate with a varying proportion.
- The Live-Dead Count is made to assess the live percentage of spermatozoa for a specific sire during that period.
- Eosin-Nigrosin stain is used for determination of live percentage. 5% Eosin and 10% Nigrosin stains are used separately.
- The time of staining is strictly 3 minutes at 35 C.
- The membrane of dead spermatozoa is permeable to the eosin so they are stained as red while the live spermatozoa remain unstained.
- To make them visible the background is stained dark with Nigrosin stain. So the live sperm are seen as white and the dead as red.

- The counting is made and reflected as percentage of the live spermatozoa in the ejaculate. The smear is prepared as follows:
- Pre-warm the microscope slides and the eosin-nigrosin stain to body temperature.
- A drop of stain is pipetted from each stain and put at the end of a slide, followed by pipetting a small droplet of semen near the stain.
- Touch edge of another slide to the drops of stain and semen – mix the semen and stain through sliding back and forth a few times; then smear the second slide over surface of the first one.
- The slide is dried rapidly through placement on a warming plate or moving it in the air, back and forth.
- Then the slide is examined using a bright field microscope (40-100X objective lens).
- The dead spermatozoa percentage may increase with prolonged sexual rest of the bull, cold shock or mild treatment in vitro.
- The minimum percentage of live spermatozoa should be 90% but it varies among laboratories.
- **Sperm morphology:** Morphological status of semen is correlated with the fertility of livestock.
- Heat stress increases the number of abnormal spermatozoa in the semen sample.
- Bull fertility declines when the incidence of abnormal spermatozoa exceeds 20%.
- Primary abnormalities arise during spermatogenesis and are associated with sperm heads and acrosomes.
- Secondary abnormalities arise after the semen leave the testis and are manifested by the presence of a drop on the mid piece of the tail or detached heads.
- Tertiary abnormalities arise after ejaculation and refer to other tail defects.
- **Supra-vital triple staining technique:** It is used permitting direct assessment of normal acrosome reaction of fixed sperm for scoring reacting spermatozoa in fresh and capacitated normal and abnormal semen.
- Take 200 µl semen, incubate at 37 °C for 15 minutes with equal volume of tryptan blue in phosphate buffer saline.
- A smear is made from undiluted or from 5-10 times diluted mixture.
- The mixture is mixed with 2 ml PBS and the suspension is allowed to sediment for 4 hours.

- The mixture is centrifuged at 400 to 1800 g for 10 minutes. The pallets are re-suspended in 2 ml PBS and again centrifuged at the same speed.
- The triple stained slides are examined at 1000 to 1250 x with a bright field microscope.
- Counting is made in duplicate for each sample and 100 spermatozoa are examined each time.
- **Computer assisted semen analysis (CASA):** The analysis provides a complete report of all physical parameters within few seconds.
- Video tape can be prepared and interpreted later on.
- Sperm concentration, motile %, sperm velocity, motility index, abnormal % and forward velocity may be determined through this method.

- **Hypo-osmotic swelling test (HOST):** It is a simple and economic test for determining functional integrity of sperm membrane, reflecting the metabolism and fertilizing capacity of the semen.
- 0.1 ml of test whole semen is taken and added with 1 ml test solution [0.5 ml of D-fructose (1.47%) and sodium citrate 2.7%)].
- Incubate at 37 °C for 40 minutes.
- Prepare slides and examine preferably under phase contrast microscope.
- The proportion of spermatozoa showing coiled tails and swollen tails above the initial incidence gives the final figure of sperm showing positive reaction to HOST.
- **Cervical mucus penetration test (CMPT):** It is based upon the penetration power of the spermatozoa through a column of the cervical mucus within a capillary tube.
- Cervical mucus is collected from a pre-ovulatory cow and placed on a clean glass slide in humid chamber at 37 °C.
- Capillary tube with inside diameter of 0.5 mm and length of 4 cm is placed upright on each sample.
- Cervical mucus is drawn into the capillary tube and one end is sealed with modeling clay.
- A putty is formed with modeling clay on a micro slide and about 0.5 ml of neat semen is taken in the reservoir thus formed.
- The mucus filled capillary tube is placed on the micro slide with open end being exposed in sperm reservoir.

- All slides are placed horizontally in moist chamber and incubated at 37 °C for 1 hour.
- The slides are than placed under microscope and the furthest penetration is noted. The length is measured in cm.
- For bull the penetration of 4 cm is poor, 11 cm fair and 24 cm good.

PREPARATION OF SEMEN EXTENDERS

Properties of Semen Extenders

- Semen of various species may be processed and stored for further utilization.
- Semen is collected, evaluated and semen extender is added according to the desired concentration.
- Property of extenders are given as under:
    - It provides nutrients for the sperm during storage.
    - It acts as a buffer for the extended semen against changes in pH.
    - It protects spermatozoa against cold shock.
    - It maintains proper osmotic pressure.
    - It contains antibiotics to inhibit bacterial growth,
    - It enhances the semen volume while maintaining its quality and viability.
    - It protects the spermatozoa from freezing and thawing shocks.

Preparation of Semen Extenders

- Ready made extenders are commercially available; alternately, ingredients may be used for preparation of semen extender according to various proved formulae.
- Many products may be used for preparing extenders for dilution of semen of various species to be stored in fresh or frozen form.
- Skimmed milk is mostly used in extenders for fresh chilled semen. For frozen semen the extenders usually contain egg yolk.
- Appropriate levels of osmolarity and pH may be maintained in the extender. Commercial extenders are also available in the market which may provide good quality semen.

PROCESSING OF SEMEN AND STORAGE

Principles

- Due to the ongoing metabolism, spermatozoa will consume the available substrates of the plasma leading to a rapid decrease in semen motility.
- To avoid such a situation, the semen should be mixed with extender after it is collected, preferably within a 15 minutes duration.
- The semen should be diluted with extender in a ratio of 1:5 up to 1:50, according to concentration of the semen.
- Final concentration of spermatozoa should be adjusted in the extended sample. The concentration of the diluted semen should be 20 million/dose for bull.
- Semen - extender dilution factor should be calculated based on the initial concentration of sperm to give a final concentration of 20 million sperm/ml for bull semen.

Example of Dilution

- Volume of semen = 10 ml, concentration = 1000 million/ml
- Desired concentration = 20 million/ml
- Dilution factor = 1000/20 = 50
- Total volume = 10 x 50 = 500 ml (semen + extender)
- Extender volume = 500 ml – 10 ml = 490 ml

Preparation and Storage of Liquid Semen

- After dilution of semen with the extender it is gradually cooled to up to 4 °C in cold handling cabinet.
- A simple method for preparing extended, chilled semen is to dilute the raw semen with warm extender in a reagent bottle, suspend the bottle in a pot with warm water at 37 °C and place the pot in a cold handling cabinet or a refrigerator.
- The water in the pot will cool slowly, thereby avoiding cold-shocking the sperm.
- Liquid semen is preserved and stored in liquid state at 4 °C.
- Liquid semen may be packed in glass vials of 5 ml in thermos flask; the vials are placed above ice cubes separated through a layer of cotton or cloth.
- Shelf life of the liquid semen is up to 4 days.
- 1 ml dose is usually used for AI.

Preparation of Frozen Semen

- After diluting the semen with proper extender in reagent bottles, the bottle is placed in a pot with water at 37 ^{0}C.
- The semen samples are tested for quality before further processing for freezing.
- The pot is placed in cold handling cabinet for slow cooling.
- After the cooling is completed, the semen is packed into semen straws (pre-sterilized and labeled) through suction pumps; the straws are sealed with polyvinyl powder and placed in the cold handling cabinet.
- The semen straws are shifted and spread on racks in a manual or computerized freezing tank.
- The semen straws are kept in the liquid nitrogen vapors for 7 minutes and then dipped into liquid nitrogen.
- Then the semen straws are packed in mini or medium sized plastic goblets and shifted to semen bank or liquid nitrogen containers.
- Following freezing of the first sample, a semen straw is thawed to determine the success of the freezing process and individual motility of spermatozoa.
- The semen motility is tested after 24 hours and recorded according before issuing for insemination.
- Bovine semen is usually processed according to standards set up by the National Association of Animal Breeders (NAAB) and Certified Semen Services (CSS).In case of preparing frozen semen, the diluted semen is held in liquid state for several hours before freezing, called as equilibration time.
- It enhances the survival of spermatozoa during freezing.
- It is usually thought to be necessary for replacement of water inside the sperm cell with glycerol.
- This process prevents formation of ice crystals inside the sperm cell and so prevents damage to the spermatozoa.

Storage of Frozen Semen

- Semen can be stored in frozen form indefinitely in liquid nitrogen at -196 °C.
- The farmer should keep a semen container at the dairy farm for insemination of cow. Cows have a short estrus and predictable ovulation.
- Frozen semen may be stored for longer periods. Higher quality is maintained for AI bulls still in production, while the progeny testing is completed.
- Capacitation gets already started in the frozen - thawed semen, therefore the sperm life span is reduced and there is a need to inseminate the cow closer to ovulation for better conception rates.

- Liquid nitrogen tank comprises two walls, one inside the other and connected at the top end. A vacume is created between the two walls and it is packed with insulation to avoid entry of heat of the inside chamber containing liquid nitrogen.
- If frost is seen on the outside of the tank, near the neck, it indicates that the tank has got damaged it has lost insulating properties.
- Avoid skidding the tank across the floor and do not bang against the wall or floor, otherwise the neck tube may get damaged.
- Liquid nitrogen keeps on evaporating and the tank will need to be refilled, arrangements may be made for periodic refills of the tanks.
- Levels of nitrogen should be checked in the tank regularly, on a monthly or weekly basis.

EVALUATION OF THAWED SEMEN

- Frozen semen is thawed and evaluated before insemination.
- The semen straw is taken out of liquid nitrogen and put in warm water at 35 °C for 30 to 60 seconds.
- The thawing technique and/or temperature vary for various protocols of freezing and packaging systems.
- Liquid nitrogen storage at -196 °C gives shelf life to the semen for an indefinite period.
- However, taking out semen from the liquid nitrogen even though still frozen, is detrimental for semen quality.
- Neck of the tank provides a temperature gradient from -196 °C to room temperature. Therefore, repeated exposure of semen at the neck will reduce semen post-thaw semen viability to a greater extent.
- Semen should be kept below the level of the neck unless it is removed from the tank. Semen be kept below the surface of the liquid nitrogen or as low as possible, in the tank.
- The goblets kept at higher levels are at more risk than lower ones if the tank is not provided with sufficient liquid nitrogen and semen is not handled properly.
- Shelf life of the frozen semen decreases after thawing.
- Semen packed in defective type of straw will break and become damaged.
- After thawing the semen straws are taken out; dried with paper towel; cut at the sealed end; a drop of semen is taken on a warm glass slide and covered with a cover-slip.

- Semen motility is recorded as percentage which must be above 40% and concentration must be above 20 million spermatozoa per dose in the case of bull.
- After thawing the semen straw must be used immediately for insemination as its viability will decrease rapidly with the passage of time.
- Thawing should only be done when the animal is ready for insemination.
- Freeze-thaw sperm survival rates vary in different males and different ejaculates from the same male.

ARTIFICIAL INSEMINATION (AI) AND CLINICAL USE OF HORMONES AND ULTRASONOGRAPHY

Artificial Insemination (Ai)

- AI is the process of introducing semen into vagina or cervix of a female by means of artificial insemination gun or rod instead of the sexual intercourse.
- AI was first introduced for animal breeding in Russia in the early 20th century; presently it is being extensively used in cows as well as other animals.
- Pregnancy may be induced in women failing to get pregnant through sexual intercourse with her partners, through the use of husband's or donor's semen.
- With the use of AI gun or AI rod the semen is placed in the female's uterus or cervix known as intrauterine or intra-cervical insemination.
- In dairy cattle the modern techniques for AI were first developed for the first time for insemination of many cows to be impregnated using bulls with better traits for milk production.
- Artificial insemination is used in domestic animals for the purpose of propagating the desirable characteristics of one sire into many dams. Additional benefits comprise overcoming breeding problems, especially in cattle, swine, swine and pedigreed dogs.
- Semen is collected from the sire, examined and extended the laboratory, following be preservation through cooling or freezing.
- The semen may be used on site of collection or may be transported to the site where the females are located.
- The frozen semen is packed in straws which are small plastic tubes.
- During the cooling and freezing process the spermatozoa are kept in a viable state through mixing with a solution containing glycerol or other cryoprotectants.

- The extender increases volume of the semen from a donor for the purpose of impregnating more females through injection of fewer sperm.
- Spread of venereal diseases is prevented through addition of antibiotics like streptomycin and penicillin to the extender.
- Presently farm animals are bred with the help of artificial insemination. Dairy cattle and swines are covered through AI to the tune of 75% and 85% of all inseminations.
- Artificial insemination helps the farmer by providing an economical means to breed their cows with males possessing the excellent traits.
- Although many personnel factors contribute to low conception rates, many producers have lost enthusiasm for AI which might be rekindled by increased success to AI.

Techniques of Ai

- Semen is injected into the female reproductive tract at proper place and optimum time for getting conception. Various techniques of insemination are described as follows:
- **Vaginal insemination:** In vaginal insemination the semen is placed in the anterior portion of vagina.
- A vaginal speculum attached supported with head light are used. Semen is injected through an insemination pipette attached with a syringe.
- Vaginal environment are not suitable for semen survival and conception rate is low in spite of a larger volume of semen used. So this method is outdated.
- **Speculum cervical method:** The speculum is lubricated with sterilized liquid paraffin and inserted into vagina.
- The insemination pipette is inserted through the speculum and guided through the head light, into the cervix.
- Draw the semen in a syringe, connect it with the pipette and deposit the semen inside the cervix.
- This method needs a good number of specula which is practically difficult.
- **Manual cervical method:** This is similar to the speculum cervical method with the exception that no speculum and headlight are used for location of the cervix and guiding the insemination pipette. Rather the hand is inserted per vagina and the cervical opening is located with the help of fingers before insemination.
- This method may lead to infection of the reproductive tract.
- **Recto-vaginal method:** This is the most widely practiced method in artificial insemination of cows and buffaloes throughout the world.

- It is preferred over other methods as it provides an opportunity to palpate the reproductive organ, confirm the status of estrus and inseminate the cow in cervix or uterus.
- The cow to be inseminated is secured in a cattle crush.
- The inseminator puts on protective clothing and gum boots and should wear full sleeved gloves, properly lubricated through liquid paraffin or toilet soup.
- His nails should be trimmed properly to avoid any injury to the rectum of the cow. Left hand (preferably) should be inserted to the rectum of the cow while right hand should be free for recording observations.
- Feces are removed by back racking and if there is contraction the hand is rested in one place. By massaging the contraction ring forward and backward, the contraction ring may be eliminated.
- Estrus is confirmed as well as non-pregnancy, before a decision is made to inseminate the animal.
- Clean and dry the vulvar lips with a cotton swab and dilate the vulva to avoid contamination of the AI pipette (AI gun or rod).
- Insemination pipette is inserted into vagina at 20-30° angles to avoid entry of the rod into the sub-urethral diverticulum and is directed to the external os of the open cervix.
- The insemination pipette should be inserted gently avoiding any trauma to the cervix.
- **Insemination with frozen semen:** AI gun is used in case of frozen semen in which the semen straw (0.5 or 0.25 ml) is thawed and dried with paper towel.
- Plunger of the AI gun is drawn outward to create space for the semen straw. The straw is put into the straw is inserted into the tip of the AI gun, with the cotton plug end pointing downward. Polyvinyl powder of the straw is cut.
- AI sheath is brought over the tip of the AI gun and pulled down up to the ring through which it is fixed. Now the gun is inserted into vagina, the hand being in rectum with the cervix grasped firmly.
- The AI gun is guided through the open end of the external os of the cervix and gently pushed forward into middle of the cervix or up to the uterus. The plunger is pushed forward to push out the semen out of the straw. The AI gun is gradually withdrawn.
- **Insemination with liquid semen:** Estrus and non-pregnancy are confirmed per rectal palpation.
- A sterilized AI rod is connected with a syringe through a rubber connector and liquid semen is taken out of the vial.
- The AI rod is inserted into the vagina as described above.

- Liquid semen is injected into the reproductive tract in cattle and buffaloes, preferably in the mid cervix.
- The AI rod is gently taken out.

Efficiency of Ai

- Efficiency of AI may be determined by measuring non-return rates and pregnancy rates as a measure of fertility.
- Non-return rates may be influenced by management factors and tend to overestimate fertility.
- Although pregnancy rates provide a more accurate measure of fertility than non-return data, pregnancy rates include fertilization success or failure, and early embryonic death.
- Consequently, pregnancy rates do not give insight into how physiologists and producers might optimize fertilization rate, and decrease the incidence of early embryonic death.
- The spermatozoa may be made accessible to ova if the animal is given deep intra-uterine insemination using this semen in a split dose with a side delivery device, in comparison to conventional uterine body artificial insemination, as measured by the competitive ratio of unmarked to marked accessory spermatozoa.
- The deep uterine insemination allows for a shift in the ratio of spermatozoa in the reservoir of the oviductal isthmus. The number of accessory spermatozoa is thought to parallel the number of potential fertilizing spermatozoa available to the ovum. Positive effect of uterine horn inseminations may be related to the minimization or elimination of cervical semen deposition. Cervical insemination errors account for approximately 20% of attempted uterine body depositions.

CLINICAL USE OF REPRODUCTIVE HORMONES

The Endocrinological Cascades

- Hormones show a varying pattern during various reproductive functions and states.
- Estrous cycle continues in post-pubertal non pregnant animals and repeats itself at an almost fixed interval through the action of prostaglandins secreted by the uterus.
- During pregnancy the progesterone levels are higher which keeps the uterus in a quiet state, preventing myometrial contractions.
- Parturition initiates at the end of gestation, resulting from increasing levels of estrogen and oxytocin and decreasing levels of progesterone.

- A single ovum is released in a monotocus animal during estrous phase of the cycle and multiple ovulation occurs in polytocus animals.
- Sufficient levels of LH are needed for ovulation, estrogen for manifestation of estrous symptoms, progesterone for pregnancy maintenance, prostaglandins for regression of corpus luteum and oxytocin for parturition.

Assisted Reproduction

- Hormones are used for treatment of various reproductive disorders, manipulation of estrus cycle and enhancement of efficiency of reproduction in domestic animals.
- Hormones in natural form or their synthetic analogues may be used for this purpose.
- Synthetic analogues of hormones are more feasible as their shelf life and half life is longer and their dose rate is lower than the natural products.
- Various commercial preparations of hormones and their synthetic analogues are enlisted in table 4.4. The dosage and indications are as recommended by the manufacturers or by oaks et al. (2001).

Table 4.4 Clinical use of reproductive hormones

Trade name	Active ingredient	Indications	Dosage
1. Gonadotrophin releasing hormone (GnRH) • Gonadotrophin Releasing Hormone (GnRH) and its analogues probably have neuroendocrine, in addition to neurotransmitter and neuromodulatory functions. • The neuroendocrine axis possesses a major role in the controlling the reproduction, and GnRH-I is integrates the internal as well as external signals of the nervous system to reproductive system. • GnRH controls release of FSH and LH from the pituitary.			
Fertagyl (Intervet)	Gonadorelin, a deca-peptide, identical to natural GnRH 0.1 mg/ml	Cattle: cystic ovaries, delayed or Anovulation, anestrus, improvement of fertility when given at AI and mid-cycle (day 11-14) and in the early post partum period (< day 40). Horse: induces ovulation.	Cattle: cystic ovaries; 0.5 mg i.m. Improves fertility when administered at AI, 0.25 - 0.5 mg i.m.; when administered, mid-cycle (day 11-14) 0.25 - 0.5 mg i.m.; when administered in the early post partum period (< day 40) 0.1 - 0.25 mg i.m.) Rabbit: induction of ovulation: 0.02 mg i.m.
Receptal (Intervet)	Buserelin acetate (0.0042 mg/ml)		Cows: Cystic ovaries with and without symptoms of nymphomania, 5.0 ml; Anoestrus 5.0 ml; Delayed ovulation 2.5 ml; Anovulation 2.5 ml; Improvement of conception rate after AI 2.5 ml; Improvement of pregnancy rate at 11-12 days post AI, 2.5 ml; Prophylaxis of fertility disorders by early induction of cycle 5.0 ml. Mares: Anoestrus 2 times 5.0 ml, repeated after 24h; Induction of ovulation 10 ml; Fixing time of ovulation and service 10 ml; Improvement of conception rate 10 ml; Prolonged or continuous estrus 10 ml. Rabbits: Induction of ovulation at post partum insemination; 0.2 ml; Improvement of conception rate 0.2 ml.

Table 4.4 Clinical use of reproductive hormones (contd.)

Trade name	Active ingredient	Indications	Dosage
2. Pregnant mare serum gonadotrophin (PMSG) • Pregnant mare serum gonadotrophin (PMSG) also known as equine chorionic gonadotrophin, eCG, has an activity which is similar to Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH). • PMSG is used in anestrus, infertility, impaired spermatogenesis and for inducing superovulation. • PMSG has been used for inducing superovulation due to its FSH like activity in domestic animals. However, due to its prolonged half life the development of follicle and ovulation prevalent over an extended period, makes it non-feasible as a superovulation hormone.			
Folligon (Intervet,	PMSG, Folligonan, Intergonan), a freeze dried product	Females: - anestrus (cows, rabbit, bitches, mink). Superovulation (for embryo transfer cows, rabbit, deer) - to improve fertility after oestrus synchronisation, to increase ovarian activity (cows, goat, sheep, deer) Males: - Impaired spermatogenesis	Cattle: Anoestrus 500 - 1,000 IU Cattle: Superovulation, 1,500 - 3,000 IU, intramuscular, day 8-13 of estrous cycle, to be followed by 3 ml Prosolvin, i. m 48 hours. Cattle: 300 - 750 IU, intramuscular, after the progestagen treatment Bitch: Anoestrus, 500 IU per animal (20 IU/kg B. wt per day for 10 days, i. m., followed by injection of 500 IU hCG at day 10. Goat, In normal and non-breeding season: After progestagen treatment 400-750 IU, intramuscular after the progestagen treatment. Rabbit, Anoestrus and super-ovulation: 40 IU, intramuscular or subcut 40 IU. Sheep, In normal and non-breeding season: After progestagen treatment, 400-750 IU, intramuscular. Mink: Anoestrus, 100 IU, intramuscular twice giving an interval of 2 days. Deer, following progestagen pre-treatment. Superovulation: 200 IU, intramuscular, 200 IU, i.m. in combination with 0.5 unit FSH

Table 4.4 Clinical use of reproductive hormones (contd.)

<table>
<tr><th>Trade name</th><th>Active ingredient</th><th>Indications</th><th>Dosage</th></tr>
<tr><td colspan="4"><u>3. Progesterone</u>
• Progesterone prepares the uterus for pregnancy, maintains the pregnancy, and inhibits the cows from showing estrous signs.
• Progesterone exerts a negative feedback effect on the hypothalamus and pituitary glands which causes cessation of folliculogenesis.
• A declining level of progesterone in case of a non-pregnant animal at the end of the diestrous period removes the negative feedback.
• Exogenous supply of progesterone for 12 days will exert the same affect and its withdrawal will result in folliculogenesis/ovulation.
The author developed a protocol for progesterone injections in buffaloes for 12 days at the rate of 50 mg on alternate days in buffaloes which resulted in induction of fertile estrus. The treatment was recommended by SAARC for field use.</td></tr>
<tr><td>Chrono -gest (goat) , Intervet</td><td>Cronolone- (45 mg fluorogestone acetate) impregnated intravaginal sponge</td><td>• Induction of synchronized estrus and ovulation.
• Controlled breeding in does.</td><td>
<table>
<tr><th colspan="2">During breeding season</th></tr>
<tr><th>Long treatment</th><th>Short treatment</th></tr>
<tr><td></td><td>Day 10: Inj Prosolvin 0.5 ml i.m.</td></tr>
<tr><td>Day 17-21: Remove sponge + Inj Folligon</td><td>Day 12: Remove sponge + Inj Folligon</td></tr>
<tr><td>Milk production
< 3.5 l/day
> 3.5 l/day</td><td>Dose of Folligon
400 IU i.m.
500 IU i.m.</td></tr>
<tr><th colspan="2">Out of breeding season</th></tr>
<tr><th>Long treatment</th><th>Short treatment</th></tr>
<tr><td>Day 0: Insert Chrono-gest sponges</td><td>Day 0: Insert Chrono-gest sponges</td></tr>
<tr><td colspan="2">Day 10 (48 hrs before removal of sponges): Inj Prosolvin 0.5 ml i.m. + Inj Folligon</td></tr>
<tr><td>Anoestrus season</td><td>Milk prod Dose Folligon
< 3.5 l/day 600 IU i.m.
> 3.5 l/day 700 IU i.m.</td></tr>
<tr><td colspan="2">Remove sponge on 21st day; mating from 36 hours after sponge removal.</td></tr>
</table>
</td></tr>
</table>

Table 4.4 Clinical use of reproductive hormones (contd.)

Trade name	Active ingredient	Indications	Dosage
Estradiol • Estradiol is an ovarian hormone produced by the developing and Graafian follicles under the influence of FSH. • The estradiol acts upon the myometrium causing its contraptions during estrus and parturition. • Estradiol is responsible for manifestation of estrous symptoms in the females. • Estradiol causes the pre-ovulatory LH surge resulting in ovulation. • Estradiol causes development of mammary glands, gametes transportation and increase resistance of the genital tract to infection during the estrus period.			
Oestradiol Benzoate (Intervet, oestradiol)	(i) Oestradiol 17b-benzoate 0.2, 1, 5 mg/ml;(ii) Arachis oil 915.8, 915, 818 mg/ml;(iii) Benzyl alcohol 104 mg/ml.	Treatment of infertility, pyometra, metritis in large animals, hormonal diseases and mesalliance in the dog, fertility management.	Species / Dose (mg/kg) / Route of administration Cow / 0.006 / Intramuscular Bitch / 0.01 / Intramuscular or subcut Contra-indications: After high doses, dogs may show blood dyscrasia. The use of Oestradiol may exacerbate mammary tumors and endometrium carcinomata. The use is contra-indicated in animals with severe liver affictions
Progesterone + hCG			
Nymfalon (Cystimeth , Intervet)	A freeze-dried, 3000 IU hCG + 125 mg progestero ne, solvent	Treatment of cystic ovarian syndrome (including nymphomania) in cattle	One vial (3000 IU hCG + 125 mg progesterone) is one dose. The freeze-dried product should be reconstituted immediately before use. The reconstituted product should be administered by slow intravenous injection. If cysts do not disappear within 10 days after treatment a second injection should be given.

Table 4.4 Clinical use of reproductive hormones (contd.)

Trade name	Active ingredient	Indications	Dosage
Prostaglandin • Prostaglandin ($PGF_{2\alpha}$) is secreted from uterus and is also is also available in synthetic form. • Prostaglandin cause regression of corpus luteum at the end of the diestrus phase of the estrous cycle.			
Prosolvin (prostaglandin nalogue, Intervet,) (Other: Estrumate, Cloprostenol, ICI).	Luprostiol (7.5 mg/ml) in 70% propylene glycol and 30% water for injection; A synthetic prostaglandin F2a analogue.	Cattle: - Sub estrus (pseudo-anoestrus): - Controlled breeding - Synchronization of estrus - Chronic endometritis, pyometra, mummified fetus - Expulsion of purulent fluid, fetus - Induction of abortion/parturition: Horse: - Luteolysis followed by fertile estrus - Second heat 20 d post-partum: - Treatment of anoestrus - Early foetal death, resorption - Induction of oestrus - Pyometra and endometritis - Induction of parturition Sheep: Planned breeding	***Dosage:*** Species Dosage Administration Cow 2 ml (15 mg) i.m. Heifer 1 ml (7.5 mg) i.m. Horse 1 ml (7.5 mg) i.m. Sheep 1 ml (7.5 mg) i.m. Pig 1 ml (7.5 mg) i.m. Following palpation of a corpus luteum during rectal examination, cows may be treated with Prosolvin and subsequently inseminated at the appearance of estrus. Animals that do not come in heat following a single injection should receive a further injection 11-13 days after the first treatment and be inseminated 72 and 96 hours later, or on detected heat. - The compound should not be given to pregnant animals unless the intention is to produce abortion or parturition.

Table 4.4 Clinical use of reproductive hormones (contd.)

Trade name	Active ingredient	Indications	Dosage
Oxytocin • Oxytocin is a peptide hormone produced by the neurons of the supra-optic nucleus and is transported to and stored in the posterior pituitary gland. • Oxytocin causes milk let down, myometrial contractions during parturition and postpartum uterine involution and during estrus period. • Synthetic oxytocin is available commercially in a highly purified form. • Aqueous extracts of mammalian pituitary glands are available which also contain other posterior pituitary hormones. • Oxytocin is used in buffaloes for milk let down in the Indo-Pak region, under field conditions.			
Oxytocin, Leo Laboratories Ltd.; Hyposton Anterior Pituitary Extract, Upjohn Ltd; Oxytocin, Intervet;		Cattle: Milk let down, hastening postpartum uterine involution following dystokia and caesarian operation, uterine trauma and hemorrhage. Horse: Induce foaling, milk let down, retained fetal membranes. Dog: Uterine inertia, retained fetal membranes, milk letdown	Cattle/Horse: 10 IU Sheep/goats: 2-5 IU; Dogs/cats: 0.5-5 IU; pigs 5 IU

ULTRASONOGRAPHY

- Ultrasonography may be defined as imaging with ultrasound. Diagnostic Ultrasonography is the cross sectional anatomic and flow imaging using pulse-echo ultrasound.
- Ultrasonography is being extensively used for monitoring infertility in cattle and buffaloes and pregnancy diagnosis/health disorders in sheep, goats, cats and dog etc.
- Ultrasonic technique is a non-invasive and painless one.

Principles

- The technique is based on difference between the acoustic impedance (resistance to transmission) of ultrasonic waves striking interfaces between media or tissue of varying composition.
- Various types of image formats can be displayed. The most familiar one (and the one that creates the actual image of anatomy) is B-mode, or grayscale scanning.
- The sound beam is produced by a transducer placed in contact with the animal. An ultra-short pulse of sound is directed into the animal, after which the transducer switches to the receive mode.
- Echoes occur as the sound beam changes velocity while passing through tissues of varying density. The greater the change in velocity, the greater the strength of the echo.
- The transducer then reconverts the echoes to electrical impulses recorded by the computer in the ultrasound machine. The strength of the echo, the time required for the echo to return after the pulse, and the direction the sound beam was sent is recorded.
- Using the information from multiple echoes, the machine creates an image that represents the appearance of the tissues when cut in that same plane on an anatomic specimen.
- In modern scanning systems, the sound beam is swept through the body many times per second, producing a dynamic, real time image that changes as the transducer is moved across the body. This real-time image is easier to interpret and allows the examiner to scan continuously until a satisfactory image is obtained.
- Trans-rectal ultrasonography can be used for diagnosis of pregnancy from 28 days, but accuracy is dependent on identification of fetal heart beats.

Procedures and Applications

- An Ultrasound machine model SSD-500 Aloka, Japan or any other suitable model, may be used for the purpose.
- The machine uses ultrasonic sound waves in the frequency range of 1.5-15 megahertz (MHz) to create images of body structures based on the pattern of echoes reflected from the tissues and organs.
- A linear probe is used per rectum for diagnosing uterine contents and ovarian status in large ruminants.
- A disc probe is used on surface of the skin to visualize the internal organs, including uterus. For diagnosing the uterine contents, the probe is applied anterior to the udders.

- In sheep and goats the external disc probe is applied on the skin cranial to the udder while they are in standing positions. Pregnancy can be diagnosed 30 days post-conception and the barren animals may be reducing the farm cost.
- In large animals the probe is inserted per rectum and placed on the uterine wall for visualization of its contents or the ovarian structures.
- The gel is applied on the probe and the probe is slowly moved on the skin surface.
- Monitoring infertility in cattle and buffaloes and pregnancy diagnosis/health disorders in sheep/goats, cats and dog etc, may be conducted.
- The technique may be successfully utilized in diagnostic and research work like folliculogenesis, follicular and luteal disorders, anovulatory estrus and fetal development pattern in large animals. In sheep and goats the status of uterus, pregnancy development and existence of twins may be investigated.

Biosafety

- If the ultrasound intensity is sufficiently high (in excess of 10 W/cm2 delivered over considerable periods of time); many deteriorating effects may result from ionizing radiation exposure.
- Chemical bonds can be disrupted, macromolecules can be degraded, chromosomal aberrations can be produced, and cells can be killed.
- Exposure of the fetus in the early stage of pregnancy can be considered a whole body exposure. Some studies with mice and rats with exceptionally high intensities of ultrasound waves have found measurable harmful effects. No such harmful human effects have been observed.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
AMH	Anti-mullerian inhibiting hormone
AMP	Adenosine monophosphate
ATP	Adenosine triphospate
bFGF	Basic fibroblast growth factor
BMP	Bone morphogenetic proteins
BSE	Breeding Soundness Examination
BTB	Blood testis barrier
CART	Cocaine- and amphetamine-regulated transcript
CASA	Computer assisted semen analysis
CL	Corpus luteum
CMPT	Cervical mucus penetration test
CRH	Corticotrophin releasing hormone
DHEA	Dehydroepiandrosterone
DHT	Dihydro-testosterone
ECF	Early conception factor
eCG	Equine chorionic gonadotrophin
EIA	Enzyme immunoassay
EM	Electron microscopy
EPF	Early pregnancy factor
ER	Endoplasmic reticulum
FSH	Follicle stimulating hormone
GAGs	Glycosaminoglycans
GDF-9	Growth and differentiation factor-9
GH	Growth hormone
GH-IH	Growth hormone inhibition hormone
GH-RH	Growth hormone releasing hormone
GnRH	Gonadotrophin releasing hormone
GV	Germinal vesicle
GVBD	Germinal vesicle breakdown
HCG	Human chorionic gonadotrophin
HOST	Hyposmotic swelling test
HPA	Hypothalamo-pituitary-adrenal axis
ICSH	Insterstitial cells stimulating hormone
IGFBP-1	Insulin-like growth factor binding protein-1
IL-1, 8	Interleukin-1, 8
LBS	Low breeding season
LH	Luteinizing hormone
LH-RH	Luteinizing hormones releasing hormone
LIF	Leukemia inhibitory factor

mRNA	Messenger RNA
MRP	Maternal recognition of pregnancy
NBS	Normal breeding season
ODF	Outer dense fibers
ODF	Oviductal fluid
PAS	Periodic acid Schiff
PDMs	Phospholipid-derived mediators
$PGF_{2\alpha}$	Prostaglandin
PIF	Prolactin inhibiting factor
PL	Placenta lactogen
PMN	Polymorphonuclear neutrophil leukocytes
PMSG	Pregnant mare serum gonadotrophin
P-Tyr	Tyrosine phosphorylation
RIA	Radioimmunoassay
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SOD	Superoxide dismutase
STH	Somato-trophic hormone
TEM	Transmission electron microscopy
TJ	Tight junctions
TRH	Thyrotrophin releasing hormone
TSH	Thyroid stimulating hormone
ZP	Zona pellucida

ABOUT THE AUTHOR

Prof Dr Muhammad Subhan Qureshi was born in District Bannu, Pakistan during 1959. After getting primary and higher secondary education in his hometown, he joined the University of Veterinary Animal Sciences Lahore during 1978 and got DVM and MSc (Hons) degrees in Animal Reproduction. He got PhD degree in Animal Reproduction during 1998 from the University of Agriculture Faisalabad. His professional career started during 1982 as Veterinary Officer at provincial Livestock Department and was selected at Veterinary Research Institute Peshawar during 1990 as Research Officer. He joined the Khyber Pukhtunkhwa Agricultural University Peshawar during 2005 as Professor.

The scientist has been working as Member Editorial Board of the Asian Australasian Journal of Animal Sciences, published from Korea, since 1999. The scientist also worked as Co-Editor of the Sarhad Journal of Agriculture and evaluated research articles for the Journal of Dairy Science USA, Canadian Journal of Animal Science and Indian Journal of Dairy Science. He has co-authored a book titled "Lactation-Reproduction Interaction in Dairy Buffaloes", published by VDM Verlag, Saarbruecken, Germany. He has published 18 research papers in journals with impact factor. In other international and national journals and conferences, he has published more than 100 papers. The number of his citations is more than 20.

Prof. Qureshi was selected as member Society of Theri ogenology USA and American Dairy Science Association. He has been assisting various universities, government departments and scientific organizations in professional assignments. He has been awarded Star Award 2008 and Star Laureate 2008 by the South Asia Publications. He was selected as visiting professor by Charles Sturt University, Australia. The appointment provides for participation and collaboration with CSU research teams in the area of stress physiology and animals' welfare. Prof. Qureshi has shared responsibility of teaching livestock management and reproduction courses to the students of Doctor of Veterinary Medicine, MSc Hons and PhD. So far, he has supervised and co-supervised 21 MSc Hons/M.Phil and 4 PhD students while thesis work of 19 students of the two degree programs is in progress. The scientist was awarded honoraria by SAARC for technology development.

He has been advising various national and international R&D organizations. Peri-urban dairy farms were studied during his doctoral research reporting huge losses. A mega Development Mission was initiated by linking livestock resources with State and the business institutions. Two successive Chief Ministers of NWFP were presented development plans which were approved. Livestock Investment Conference was organized in collaboration with Sarhad Chamber of Commerce and Industries in 2005 recommending establishment of diary colonies and other ventures through public private partnership. Agribusiness Support Fund was convinced to support stakeholders' training in dairy technology at the University.

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